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Leidos Biomedical Research, Inc.

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Frederick National Laboratory for Cancer Research

2015–2016 ANNUAL REPORT

Leidos Biomedical Research, Inc.

Operations and Technical Support Contractor for the Frederick National Laboratory for Cancer Research

2015–2016 Annual Report

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P. O. Box B, Frederick, Maryland 21702-1201

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2015–2016 Annual Report

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Leidos Biomedical Research, Inc.
Contract Year 8
September 26, 2015–September 25, 2016
Executive Summary

Leidos Biomedical Research, Inc. (Leidos Biomed) is pleased to submit this annual report for the Frederick National Laboratory for Cancer Research (FNLCR) Operations and Technical Support (OTS) Contract for the period of September 26, 2015, to September 25, 2016.

FNLCR is a Federally Funded Research and Development Center (FFRDC) sponsored by the National Cancer Institute (NCI). It is the only FFRDC dedicated to biomedical research. Through its status as an FFRDC, FNLCR provides NCI and others with a unique national resource to accelerate the development and delivery of effective preventive, diagnostic, and therapeutic products to people living with cancer and HIV/AIDS.

The following annual report documents the extensive breadth of activities performed by Leidos Biomed in support of NCI's mission. These activities span the research and development spectrum, including investigator-initiated, hypothesis-driven research into cancer and AIDS; advanced technology programs focused on genetics and genomics, proteins and proteomics, imaging, nanotechnology, bioinformatics, and laboratory animal sciences; clinical operations in support of NCI– and National Institute of Allergy and Infectious Diseases (NIAID)–sponsored clinical trials, as well as NCI drug discovery and development efforts; and management and operations of biopharmaceutical development and manufacturing programs under current Good Manufacturing Practices conditions for NCI and NIAID. Administrative, financial, safety, and facilities support is provided to these research and development activities through state-of-the-art business processes.

National Resource

To more effectively portray the scope of activities conducted at FNLCR, the annual report is structured to align with the institutes and agencies that FNLCR supports, as well as national mission research projects that NCI assigns to FNLCR. The breadth of activities conducted in support of its customers underscores FNLCR's role as a national resource.

FNLCR Customers

FNLCR provides direct program support to numerous divisions, offices, and centers within NCI. These include:

- **Center for Strategic Scientific Initiatives** – creates and implements exploratory programs focused on emerging scientific discoveries and innovative technologies to accelerate the pace of cancer research and the translation of research results into new therapies, diagnostics, and preventive agents.
- **Center for Cancer Genomics** – unifies NCI's activities in cancer genomics by aiming to synthesize research in different fields of cancer genomics—structural, functional, and computational—in order to improve patient outcomes.
- **Center for Global Health** – provides assistance and guidance to nations as they develop and implement cancer control plans; trains international investigators; and strengthens U.S. national, regional, multilateral, and bilateral collaboration in health research, cancer research, and cancer control to advance global cancer research, build expertise, and reduce cancer deaths worldwide.
- **Center for Biomedical Informatics and Information Technology** – collaborates across NCI to plan, provide, and coordinate technology, standards, and scientific computing in support of the NCI mission to speed discovery, facilitate open science, and progress towards precision treatment in cancer care and a learning health care system.
- **Coordinating Center for Clinical Trials** – facilitates efforts across NCI to enhance the effectiveness of NCI's clinical trials enterprise through collaboration and harmonization among NCI programs and extramural stakeholder communities.
- **Center for Cancer Research** – a productive community of NCI intramural basic researchers, clinicians, and translational scientists who integrate basic and clinical research discovery to develop novel therapeutic interventions that better treat adults and children living with cancer or HIV/AIDS.
- **Division of Cancer Epidemiology and Genetics** – conducts population and multidisciplinary research to discover the genetic and environmental causes of cancer and ways to prevent it.
- **Division of Cancer Biology** – encourages and facilitates continued support of basic research in all areas of cancer biology to provide the research foundation that enables improved understanding of the disease and may lead to new approaches for prevention, diagnosis, and treatment.

- **Division of Cancer Prevention** – conducts and supports research to find ways to prevent and detect cancer, and to prevent or relieve symptoms from cancer and its treatments.
- **Division of Cancer Treatment and Diagnosis** – supports the translation of promising research into clinical applications to improve the diagnosis and treatment of cancer in areas of unmet need that are often too risky or difficult for industry or academia to develop alone.
- **Division of Cancer Control and Population Sciences** – supports an integrated program of genetic, epidemiologic, behavioral, social, applied, and surveillance cancer research to reduce risk, incidence, and death from cancer, as well as to enhance the quality of life for cancer survivors.

FNLCR also provides significant support to NIAID. Support is provided to the following NIAID divisions/centers:

- **Division of Intramural Research** – conducts basic and clinical research in a wide range of disciplines related to immunology, allergies, and infectious diseases.
- **Division of Clinical Research** – provides multidisciplinary trans-NIAID services for facilitating clinical research and managing special projects as directed by NIAID leadership.
- **Division of Acquired Immunodeficiency Syndrome** – supports a global research portfolio on HIV/AIDS and its related co-infections and co-morbidities.
- **Vaccine Research Center** – conducts research that facilitates the development of effective vaccines for human disease.

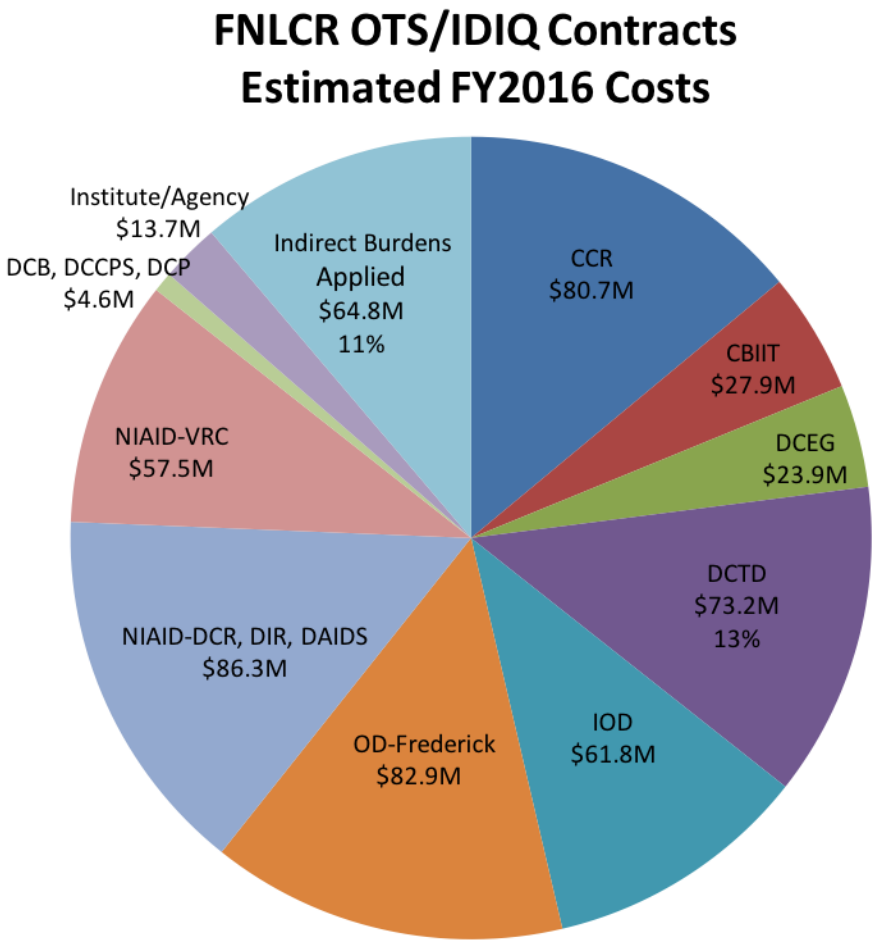
Support is also provided to approximately 15 other institutes within the National Institutes of Health (NIH) and other federal agencies.

FNLCR Customer Funding

An Indefinite Delivery Indefinite Quantity (IDIQ) contract was awarded in FY2016, which prompted cost accounting changes to realign costs from direct costs under OTS to indirect costs across multiple contracts, which is represented in FY2016. The estimated FY2016 cost for the OTS and IDIQ contracts is $577.4 million. The ARRA or Stimulus funding expired in FY2015, and the FY2016 Estimate does not reflect any costs associated with those programs.

As represented in the pie chart for the FY2016 Estimate, and when excluding Indirect Burdens Applied, 69 percent of the costs are associated with NCI programs, 28 percent associated with NIAID/VRC programs, and 3 percent associated with other Institutes and Agencies. Inclusive of Indirect Burdens Applied, the overall estimate for FY2016 represents an increase of $60.8 million or 12 percent, which reflects the growth due to the awarding of the IDIQ contract.

Since FY2014, and when excluding the Indirect Burdens Applied and Stimulus, the OTS and IDIQ contracts have increased by 13 percent in annual costs, or $57.9 million. The NCI has seen a modest growth of 8 percent, while non-NCI programs have been the major contributor to growth at 24 percent.



FNLCR National Mission

FNLCR is an integral part of an aggressive national initiative to further scientific understanding of cancers driven by mutations of the *RAS* family of genes. Established by NCI, the RAS Initiative seeks to facilitate connections between and among researchers, bringing new ideas and technologies to bear on RAS.

FNLCR serves as the research hub that connects RAS researchers, nationally and internationally, through collaborations and several spoke projects. RAS Initiative hub research areas include Structural Biology and Biochemistry, RAS Assays, Biology of Mutant KRAS Cell Lines, Pathway Analysis, Cell Surface Analysis, and RAS Reference Reagents.

This year, the National Cancer Institute, NIH has committed to establishing a national cancer research community–accessible Cryo-EM user facility that will be managed and operated by FNLCR. The purpose of the facility is to enable the collection of high quality cryo-EM images, with minimal delay between request for access and data collection.

FNLCR Organization

President's Office: David Heimbrook, Ph.D., President, Leidos Biomedical Research, Inc., and Laboratory Director, FNLCR

The three Leidos Biomed key staff report to Dr. David Heimbrook and are each responsible for leading one of the three operating groups within the OTS Contract. Within the groups there are 15 directorates, each of which has either a primary technology focus or is aligned with a primary customer. Support to the federal customers and performance of the RAS national mission are accomplished through the collaboration of these 15 directorates.

Science and Technology Group: David Heimbrook, Ph.D., Chief Science Officer, Interim

The Science and Technology Group (STG) provides scientific expertise and support for basic and applied research and data management. STG comprises the following five directorates:

- **AIDS and Cancer Virus Program (ACVP)** – pursues studies that have direct or potential relevance to the overall goal of developing an effective vaccine or other approaches for the prevention or treatment of HIV infection and AIDS, and to the study of viruses involved in cancer.
- **Basic Science Program (BSP)** – covers a wide spectrum of research activities, with a focus on immunology and genetics in support of the Center for Cancer Research.
- **Cancer Research Technology Program (CRTP)** – serves as the program hub for the RAS Initiative and provides expertise in genomics, proteomics, imaging, informatics, and nanotechnology to NCI and external partners.
- **Laboratory Animal Sciences Program (LASP)** – provides an integrated portfolio of research animal programs, including the development of genetically engineered mouse models, cryopreservation and assisted reproduction, pathology and histotechnology, small animal imaging, molecular diagnostics, and animal husbandry.
- **Data Science and Information Technology Program (DSITP)** – develops and maintains an enterprise approach to IT infrastructure support at FNLCR and operates the Advanced Biomedical Computing Center.

Clinical Group: Barry Gause, M.D., Chief Medical Officer

The Clinical Group is composed of four directorates at the clinical end of the research spectrum that are directly involved with patients. This group includes the following directorates:

- **Clinical Research Program Directorate (CRD)** – provides clinical research management, regulatory, and pharmacovigilance support to NCI, NIAID, and other NIH clinical programs and provides quality assurance for the Biopharmaceutical Development Program and the Vaccine Clinical Materials Program.
- **Applied and Developmental Research Directorate (ADRD)** – provides clinical and biological monitoring, regulatory support, biospecimen processing and storage, assay development, and project management support to NCI and NIAID clinical programs.
- **Vaccine Clinical Materials Program (VCMP)** – manufactures and provides biological agents used in NIAID-sponsored clinical trials.
- **Biopharmaceutical Development Program (BDP)** – manufactures and provides biological agents used in NCI clinical trials.

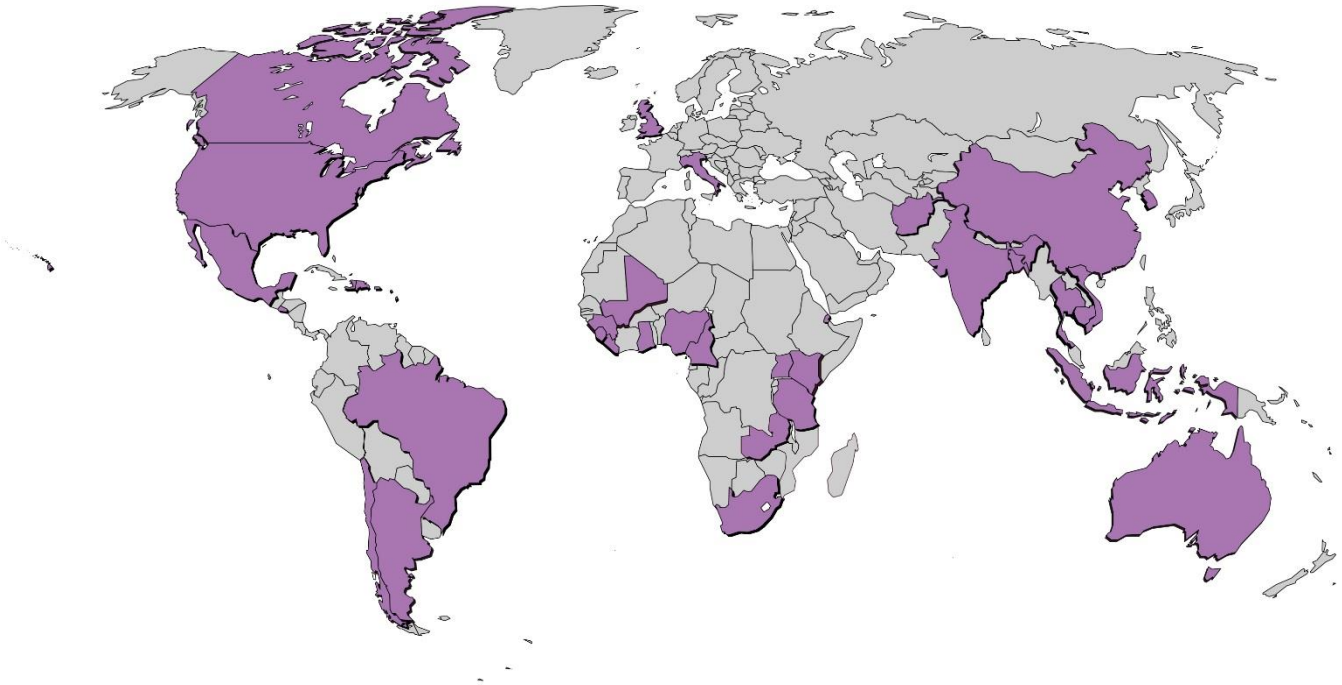
Operations Group: Kathy Terlesky, Ph.D., Chief Operating Officer, Interim

The Operations Group provides the administrative, financial, and facility operations resources required to support the myriad of research activities conducted at FNLCR. The group includes the following directorates:

- Management Support Directorate
- Contracts and Acquisitions Directorate
- Human Resources Directorate
- Facilities Maintenance and Engineering Directorate
- Environment, Health, and Safety Directorate
- Financial Operations Directorate

Global Impact

Through its direct support of clinical research, FNLCR activities are not limited to national programs. Leidos Biomed provides clinical trials management support to the NIH Clinical Center, NCI, NIAID, and several other institutes within NIH and is actively involved in more than 400 domestic and international studies related to cancer; influenza, HIV, Ebola, and other infectious diseases (such as Zika, hepatitis C virus, tuberculosis, and malaria); heart, lung, and blood diseases; parasitic infections; and rheumatic and inflammatory diseases. FNLCR provides medical and clinical research professionals to support numerous NIH clinics and has a global impact by facilitating the conduct of clinical research programs and initiatives such as the NCI Center for Global Health, NIAID's Southeast Asia Influenza Clinical Research Network, and the International Network for Strategic Initiatives in Global HIV.



Community Involvement

FNLCR is dedicated to biomedical research on a national and global level, and it approaches local community involvement with a similar passion. As an organization, Leidos Biomed is actively involved in nurturing the community in which its employees live and work. The company monetarily supports a wide cross-section of nonprofit organizations and charity fundraisers. Leidos Biomed representatives serve leadership and supporting roles in advancing local business and economic development, and are committed to being actively involved at the city, county, and state levels. FNLCR sponsors a series of educational programs supporting students from grade school through grad school, including outreach to local elementary schools, scholarship support to high school students interested in science, technology, engineering, and mathematics (STEM) careers, and advisory support to local higher education institutions.

Leidos Biomed employees are equally generous, through participation in a company program in which they make charitable contributions and provide hands-on volunteering at local organizations such as Habitat for Humanity and the Frederick Rescue Mission.

Whether through local involvement or support of international clinical trials, the staff of Leidos Biomed is committed to promoting FNLCR and helping NCI prevent, diagnose, and treat cancer and HIV/AIDS. It is a privilege to serve as NCI's FFRDC contractor in support of this important mission.

Scientific Program Support

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Leidos Biomedical Research, Inc.

SCIENTIFIC PROGRAM SUPPORT

NCI OFFICE OF THE DIRECTOR

Frederick National Laboratory

RAS Program

Support Provided by the Cancer Research Technology Program

Structural and biophysical characterization of KRAS

Within the RAS Initiative, the initial goal of structural biology efforts is to provide detailed structural insights of wild-type (WT) and oncogenic mutants of KRAS4b in the active (GTP-bound) state. The long-term goal of our efforts is to provide a molecular basis of KRAS interaction with various effectors, regulatory proteins, and trafficking proteins. The structural information obtained from these studies will not only improve our understanding of how KRAS interacts with these proteins but could also act as a blueprint for structure-based drug design.

Significant progress has been made towards obtaining structural information of oncogenic mutants of KRAS4b in active state and in complex with prenyl binding protein PDEδ and effector protein c-Raf. We continue to make efforts to crystallize KRAS4b-calmodulin (CaM) complex.

WT and oncogenic mutants of KRAS4b in the active state: To understand how different oncogenic mutations in KRAS affect intrinsic and GTPase Activating Protein (GAP)-stimulated GTP hydrolysis, we solved the structures of WT as well as G12, G13, and Q61 mutants in complex with GMPPNP (non-hydrolysable analog of GTP) and Mg^{2+}. Analysis of these structures provides a rationale for the loss of intrinsic as well as GAP-mediated GTP hydrolysis in these mutants. Mutated residues at G12 and G13 positions are likely to sterically clash with the arginine (Arg) finger, a GAP residue proposed to stabilize the developing negative charges on γ-GTP in the transition state and thereby catalyze the reaction. Even though mutated residues at Q61 position do not sterically clash with the Arg finger, the absence of glutamine (catalytic) residue results in significantly reduced intrinsic as well as GAP-stimulated GTP hydrolysis.

KRAS4b-PDEδ complex: Phosphodiesterase-δ (PDEδ, also known as PDE6δ, PrBP/δ, and PDE6D) has been implicated in membrane release and localization of prenylated RAS and other prenylated proteins. Despite significant progress made in the last few years in targeting PDEδ for KRAS-driven cancers, there was no structural information available on fully processed KRAS4b in complex with PDEδ. Last year, we solved the structures of farnesylated-methylated KRAS4b in complex with PDEδ in two different crystal forms. Our structures provide atomic details of the hypervariable region (HVR) of KRAS4b in complex with PDEδ for the first time. Comparison of the two crystal forms not only describes how farnesylated-methylated KRAS4b binds to PDEδ, but also suggests how the binding of prenylated KRAS4b and PDEδ is likely to be facilitated by a five amino acid–long sequence motif present in the HVR of KRAS4b. The conserved sequence of this motif may account for the binding of PDEδ to both farnesylated and geranyl-geranylated KRAS4b. Structure-based mutational studies suggest key roles played by various amino acids involved in the KRAS4b-PDEδ interaction. Our binding studies show that the carboxymethyl group present on farnesylated C185 plays a key role in the KRAS4b-PDEδ interaction. Structure and sequence analysis of various prenylated proteins that have been previously tested for binding to PDEδ provides a rationale for why some prenylated proteins such as KRAS4a, RalA, RalB, and Rac1 do not bind to PDEδ. Comparison of all four available structures of PDEδ complexed with various prenylated proteins/peptides shows the presence of additional interactions due to a larger protein–protein interaction interface in KRAS4b-PDEδ complex that can potentially be exploited for designing an inhibitor with minimal off-target effects.

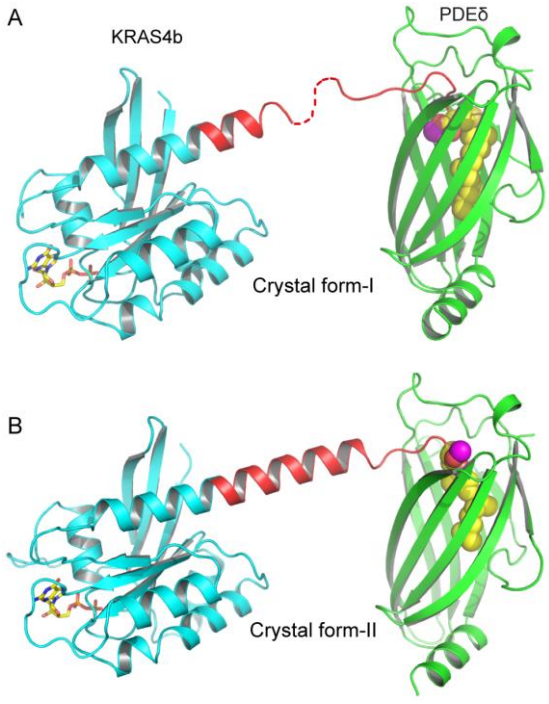


Figure 1: Overall structure of KRAS4b-PDEδ complex in two different crystal forms. Ribbon representation of guanosine diphosphate–bound fully processed KRAS4b in complex with PDEδ in crystal form I (A) and in crystal form II (B).

The PDEδ, GTPase domain, and HVR of KRAS4b are shown in green, cyan, and red, respectively. The farnesyl chain is shown as a sphere and colored yellow. The carbon and oxygen atoms of the carboxymethyl group are colored magenta and red, respectively. GDP is shown as a stick and colored yellow (carbon), and red (oxygen).

KRAS4b-CaM complex: Recent studies have shown KRAS suppresses the Wnt/Ca^{2+} signaling pathway by directly binding with **CaM** and that the KRAS4b-**CaM** interaction is attenuated by phosphorylation of KRAS4b S181 by protein kinase C (PKC). To gain structural insights on how KRAS4b interacts with **CaM**, we have been trying to solve the structure of KRAS4b-**CaM** complex at the Frederick National Lab for Cancer Research (FNLCR)/Leidos Biomed as well as through collaboration with Dr. Carla Mattos (Northeastern University). Our initial efforts to co-crystallize GMPPNP-bound KRAS4b in complex with CaM as well as fusion protein constructs consisting of CaM linked to HVR of KRAS4b via 6 or 12 amino acid-long linker failed to give any protein-protein complex crystal. Additional binding studies showed that farnesylated and methylated KRAS4b binds stronger with CaM compared to the non-processed KRAS4b. Based on this observation, current efforts are focused on the crystallization of fully processed KRAS4b (GMPPNP and Mg bound) in complex with CaM. In addition to using crystallography, we have a collaboration with Dr. David Weber (University of Maryland, Baltimore) to use nuclear magnetic resonance (NMR) to solve the structure of KRAS bound to CaM. Recent data indicates that chemical shifts are observed on ^{15}N labeled CaM with KRAS-GppNHp, but not with KRAS-GDP.

KRAS4b-Raf complex: In the active state, KRAS interacts with various effector proteins such as Raf kinase, PI3K, and Ral-GDS, leading to activation of several signaling cascades within the cell. The Raf kinase inhibitors are promising anticancer agents because they may be effective in tumors with constitutively active or aberrant Ras or Raf signaling. Despite our best efforts, we have only been able to express and purify the RAS binding domain (RBD) of Raf1(c-Raf) for structural and biophysical studies. Recently, we have solved the structure of WT as well as Q61 mutants of KRAS4b in complex with Raf-RBD. Analysis of these structures provides molecular details of the protein-protein interface and explains how Q61 mutation that affects intrinsic and GAP-stimulated GTP hydrolysis binds to Raf-RBD a like WT protein. Future efforts will focus on crystallizing G12 and G13 mutants of KRAS4b in complex with Raf-RBD.

KRAS-membrane interactions: KRAS is localized at the inner leaflet of the plasma membrane through a farnesylated and methylated terminal cysteine residue and a stretch of positively charged lysine residues located within the C-terminal HVR. While soluble KRAS can bind tightly to RAF kinase, it is only when it is bound to the membrane that it activates RAF kinase. Understanding the molecular details of how KRAS activates RAF kinase at the plasma membrane could lead to novel opportunities for drug discovery. We have focused our attention on using a variety of biophysical and structural approaches to investigate the activity and orientation of recombinant farnesylated and methylated KRAS (KRAS-FMe) on artificial membrane systems. In our earlier work we have demonstrated that KRAS-FMe interacts with lipid Nanodiscs in an anionic (phosphotidyl-serine) lipid-dependent manner.

In a collaboration with Dr. Marco Tonelli and colleagues (National Magnet Resource Facility at Madison [NMRFAM]), we have used NMR to measure structural changes of KRAS-FMe bound to lipid Nanodiscs. In these studies,^{15}N labeled KRAS-FMe expressed and purified from insect cells was combined with Nanodiscs composed of 70% palmitoly-2-oleoyl-glycero-3-phophocholine (POPC) and 30% palmitoly-2-oleoyl-glycero-3-phophoserine (POPS). The NMR data indicates that while KRAS-FMe is bound to Nanodiscs, it appears to rotate independently of the Nanodisc. Consistent with this observation, neutron reflectivity measurements performed in collaboration with Dr. Frank Heinrich (National Institute of Standards and Technology [NIST]) using tethered lipid bilayers composed of 70% POPC and 30% POPS indicated that KRAS-FMe was perpendicular to the bilayer at a distance of 30 Å. The orientation of KRAS-FMe to the membrane was independent of the nucleotide state of KRAS. In order to identify which regions of KRAS-FMe are in closer proximity to the membrane, we used a technique called Paramagnetic Relaxation Enhancement (PRE)-NMR. In this work, Nanodiscs are prepared containing a lipid chelated with a paramagnetic metal (gadolinium), which increases the relaxation of the NMR signal of the ^{15}N-label when it is within a distance of 10–20 Å, although these measurements are complicated by the fact that the gadolinium is attached to a fluid lipid membrane, so it is not in a fixed position. We performed PRE-NMR measurements of KRAS-FMe in either the GDP or GppNHp state complexed with Nanodiscs and were able to identify which regions on KRAS are closest to the membrane. The overall orientation of KRAS in either the GDP or GppNHp state was very similar, consistent with the neutron reflectivity measurements. Decreases in the NMR signal indicate those amino acids that are closer to the membrane. The PRE data indicate that the N-terminus in the beta strands 1–3 and alpha helix 5 comprise a region of KRAS that is close to the membrane. However, alpha helices 3–5 also forms another region of KRAS that is orientated towards the membrane. These data, coupled with the neutron reflectivity measurements, allow us to propose a model of KRAS that is attached to the membrane by the flexible HVR linker. However, the beta strand region and alpha helical region move back and forth in closer orientation to the membrane. Future studies will focus on how domains of the RAF kinase affect the orientation of KRAS on the membrane.

In collaborative studies with other investigators, we have explored the role that different lipids may play in the interaction between KRAS-FMe and the membrane. Dr. Stephen Sligar's laboratory at the University of

Illinois at Urbana-Champaign developed a phase-modulated fluorescence anisotropy assay to measure the interaction of KRAS-FMe and Nanodiscs. Using this assay, they demonstrated that the inclusion of phosphatidylinositol bisphosphate (PIP2) increased the binding affinity of KRAS-FMe to the Nanodiscs by almost 10-fold over Nanodiscs containing phosphatidyl-serine at a similar charge density. In a collaboration with Dr. Jay Groves (University of California, Berkeley), we investigated the behavior of KRAS-FMe in supported bilayers using a variety of different single molecule fluorescence techniques. Dr. Groves' lab showed that the addition of PIP2 in supported bilayers decreased the mobility (translational diffusion time) of KRAS compared to bilayers containing other anionic lipids. Taken together, these data indicate that KRAS may interact with PIP2 in membranes differently than other negatively changed lipids. Over the coming year, we will perform more experiments to explore these results.

Identification of small molecule-KRAS binders: In our efforts to crystalize KRAS, we obtained a crystal form where the flexible switch 1 region was in an extended conformation. This conformation had not been identified before and indicated that the switch 1 region was more flexible than previously appreciated. In addition, there was a portion of the protein that was accessible in this hinge region (Figure 1). We established a contractor Collaborative Research and Development Agreement (cCRADA) with Dr. Brian Shoichet's laboratory (University of California, San Francisco [UCSF]) to perform in silico docking experiments to identify compounds that may bind in this region. Twenty-one compounds were identified as putative binders from the screen. These compounds were obtained and their direct binding to KRAS was measured using surface plasmon resonance spectroscopy. Eight compounds were identified as KRAS binders and of these, three were confirmed to bind in the hinge region by NMR. Commercially available analogs of these binders will be purchased, evaluated for binding affinity to KRAS, and potentially used for structural studies.

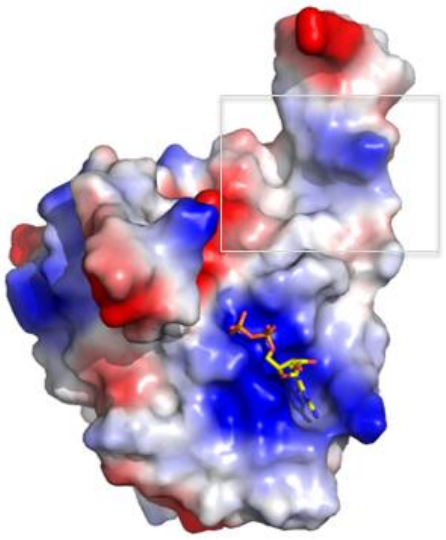


Figure 1: Crystal structure of KRAS showing the switch 1 region in an extended conformation. Residues with a negative, neutral, or positive charge are represented as red, white, or blue in this electrostatic surface representation. Compounds were docked in the in silico screen in the hinge region identified in the boxed area.

Identification of compounds that inhibit KRAS-driven tumors

This is an effort by several groups within the Cancer Research Technology Program (CRTP) to establish cell-based, high-throughput screening assays to identify candidates for therapeutic development against KRAS-driven tumors. The short-term objective is to qualify the cell-based assays within FNLCR, and then transition them to collaborators with chemical libraries and automation for the execution of screening campaigns. Hits derived from these screens will be evaluated within the laboratories at FNLCR and, when warranted, advanced to chemical optimization programs with our screening partners or additional collaborating laboratories.

Several genetically engineered systems were developed during FY2015 using the RAS-dependent mouse embryonic fibroblast (MEF) cells derived in the laboratory of Dr. Mariano Barbacid. Development and characterization of these lines continued into FY2016, resulting in a complete panel of isogenic MEF lines, each dependent on a single RAS isoform or allele for activation of the MAPK pathway and cell cycle progression.

Use of these cell lines for high-throughput screening was optimized in collaboration with the National Center for Advancing Translational Sciences (NCATS), where multiple cell lines were tested for sensitivity to a library of approximately 2,000 tool compounds and U.S. Food and Drug Administration (FDA)-approved drugs with known cellular targets. One class of compounds has been identified, which shows potent inhibition of growth in MEF cells harboring oncogenic KRAS, but relatively inactive in MEF cells with WT KRAS. The team is currently investigating the molecular mechanism behind this observation.

Several CRADAs have been negotiated with pharmaceutical companies for screening collaborations using the panel of MEF lines. A CRADAwith Sanofi is designed to identify novel compounds that inhibit the growth of a KRAS 4bG12D MEF and then use WT HRAS as a negative control to identify selective inhibitors of KRAS 4bG12D-dependent growth. Initial pilot and primary screening was performed at Sanofi in Strasbourg, France. The collaborators at Sanofi were able to optimize screening conditions for each cell line, and pilot screening revealed a molecular target that may be required for oncogenic but not WT KRAS. Compounds with differential activity will be sent to FNLCR for replication and validation. High-throughput screening will continue at Sanofi using a novel compound library of up to 1 million compounds.

Another CRADA was established in FY2016 with Tosk Pharmaceuticals, which identified a series of compounds that inhibit growth and MAPK signaling in KRAS-mutated cancer cell lines. The scope of the collaboration was to identify the molecular mechanism of action and develop a biochemical assay that could be used to develop a lead clinical drug candidate. At FNLCR, the project contributors showed that the compounds inhibit the MAPK pathway and growth in KRAS G12V MEF cells, but had little to no effect in cells harboring BRAF V600E, suggesting that the activity of the compound is KRAS-dependent.

A CRADA was established with Daiichi Sankyo in FY2015 to identify compounds that inhibit KRAS-RAF interactions using an assay developed at FNLCR. Approximately 30,000 compounds were screened at FNLCR. Hit compounds were validated at FNLCR by generating dose-response curves in orthogonal KRAS-RAF binding assays, and direct KRAS binding compounds were identified by Daiichi Sankyo through surface plasmon resonance. One chemical series was identified at the end of the collaboration, which was composed of three structurally related compounds that demonstrated selective inhibition of KRAS-RAF binding in the approximately 100 μM range. FNLCR is currently resynthesizing the compounds to validate activity and attempt to identify a discrete binding site on KRAS protein.

Characterize and disrupt KRAS complexes and probe the nature of KRAS dimerization

The goal of the RAS Imaging group is to characterize and disrupt KRAS complexes and probe the nature of KRAS oligomerization. To that end, we have developed screening assays, and conducted dynamic, single molecule studies of RAS mobility in the plasma membrane of cells. The high content assay is designed to screen for molecules that differentially disrupt the plasma membrane localization of mutant GFP-KRAS4b (Figure 1 below). It utilizes a tet-on inducible system for stable and tunable expression of GFP-tagged KRAS4b and GFP-HRAS in HeLa cells. Using this assay, we are currently conducting a screen in partnership with Eli Lilly to identify molecules that differentially perturb KRAS4bG12D plasma membrane localization.

A second assay we have developed is a Bioluminescence Resonance Energy Transfer (BRET) assay for interrogating RAS interactions in live cells (Figure 2). This assay reliably measures RAS interactions with RAF and RalGDS, and we have successfully used it to interrogate the effects of mutations in the Switch 1 domain of RAS or the RBD domain of RalGDS and RAF on these interactions. Furthermore, it is suitable for high throughput screening. We have also used this nanometer proximity based assay, to investigate the hypothesis that RAS multimerizes in the plasma membrane of cells.

To better characterize RAS complex formation in cell membranes, we have conducted a variety of Single-Molecule Studies using advanced optical microscopy and image analysis techniques (Figure 3). From single-molecule tracking of Halo-tagged RAS and RAS-associated proteins in the membrane of cells (such as Halo-KRas4b-dependent MEFS), we derived a three-component model characterizing different mobility species of KRAS4b in cell membranes. We have extended these observations, and characterized the contributions of the different domains of RAS—the effector binding Globular domain and the membrane binding Hypervariable Region—to its mobility.

In addition to the imaging assays, we have established that the RAS-dependent MEFS can be used as a primary screen to evaluate natural products. A cCRADA with Pharma Arava was signed, and we are now screening natural products against RAS-dependent MEFS to identify compounds with KRAS4bG12D selective activity (Figure 4).

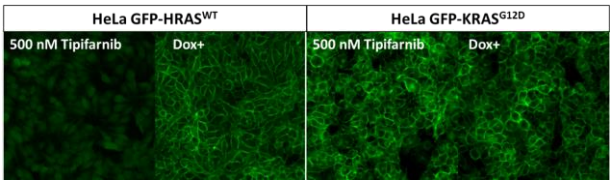


Figure 1: Confocal images of cells the GFP-HRAS^{WT} and GFP-KRAS^{G12D} cell lines. Tipifarnib is a farnesyl transferase inhibitor. Because KRAS can bypass the inhibitory effect of this molecule, Tipifarnib has no effect on KRAS localization to the membrane. HRAS, on the other hand, is highly sensitive to the drug, and mislocalization of GFP-HRAS is readily apparent in the treated cells.

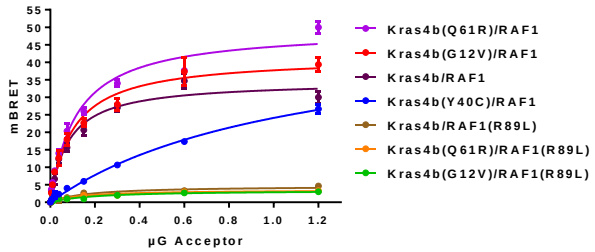


Figure 2: Donor saturation curves measuring membrane protein–protein interaction of RAS and its major effector RAF in live cells. Oncogenic variants of RAS show higher-affinity RAF than the WT protein. As expected, the switch 1 KRAS mutant Y40C shows less interaction with RAF. RAF probes with mutations in their RAS binding domains show no interaction.

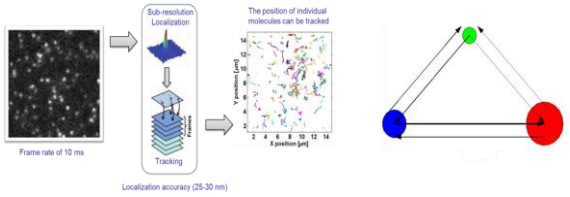


Figure 3: Individual trajectories in single-molecule tracking experiments of Halo-tagged RAS molecules were analyzed by Hidden Markov Modeling (right diagram) and showed that KRAS4b diffuses at three different rates in the membrane of live cells—a fast component (red circle), an intermediate component (blue), and a slow component (green). The arrows between the circles indicate the transition probabilities. The transition between the slow and fast mobility species is rare, indicating that there is a preferred path for molecules to transition between mobility states.

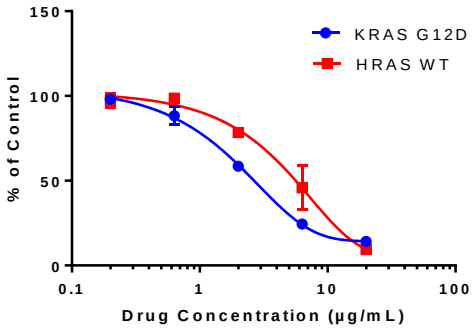


Figure 4: Dose response curves for RAS-dependent MEFS treated with increasing concentrations of a plant extract from the Israeli Desert collected by Pharma Arava. This particular extract shows a modest selectivity for the KRAS4bG12D dependent MEF.

Cell surface mapping

The aim of the cell-surface mapping project is to investigate the possibility of either unique or overexpressed proteins in KRAS tumors as compared to normal tissue. This has been performed using mass spectrometry interrogation of cell-surface protein-enhanced preparations of cell lines progressing to tumor tissue.

The investigation of the cell surface by mass spectrometry has been completed. We have not only completed the mapping of several KRAS mutant cell lines, both induced (MCF10a EV and MCF10aKRAS) and natural (H2122, H2444, SW620, A549, and KP3), but also tumors from a GEM model (provided by Dr. Barbacid) and A549 tumors. In particular, the A549 tumors have given significant information. It was found that our glyco enrichment process gave peptides that were species-specific. We have completed mass spec analysis of A549 lung tumors, adjacent tissue, and normal lung tissue isolated from litter mates. The proteins identified in the adjacent tissue could be traced to the A549 cells (human) or upregulated or induced proteins in response to the tumor (mouse). This information was presented at the American Association of Cancer Research (AACR) meeting in a poster and is the basis of a second manuscript in progress.

All of the data from all of the above experiments has been uploaded into a portal at NIST that will release the data to the public on publication. We have provided the data to three collaborators: Dr. Jim Wells at UCSF, who provided the MCF10a cells; Dr. Rob Rottapel at the University of Toronto, who is interested in comparison with his genomic analysis of pancreatic tumors; and Dr. Don Johann at the University of Arkansas, who leads a bioinformatics initiative specializing in proteomics and genomics. To date, we have completed a draft manuscript with Dr. Wells' lab that is in revision with internal authors before submission. The journal that we will submit to is Oncotarget. At this point, project efforts have been reduced to generating papers for publication.

Synthetic lethal screens

The KRAS Synthetic Lethality Workshop held at the FNLCR in January 2014 resulted in a Funding Opportunity Announement (FOA) for U01 grants for "New Approaches to Synthetic Lethality for Mutant KRas-Dependent Cancers." Six teams have been funded and, per the U01 format, a RAS Synthetic Lethality Network (RSLN) has been formed comprising the leaders of the six teams. The RSLN met at the ATRF in December 2015 and will meet again August 11, 2016.

Two websites to facilitate communications with the RAS community have been established. Cancer.Gov/RAS is primarily an outward-facing, publicly accessible site and is managed by the FNL in coordination with the NCI Office of Communications and Public Liaison. Cancer.Gov/RAS has 3,000–5,000 unique visitors each month. RAS Lab is a private, NCI-approved site that is

accessible by invitation only. All members (about 600) can post, upload, and comment. Since its inception in February 2015, there have been 151 online discussions on the RAS Lab.

The RAS Initiative has developed and published a method to purify full-length, fully-modified KRAS protein. This critical resource has not been available previously, and the KRAS Protein Purification Workshop was held at the FNLCR in May 2016 to help ensure that outside laboratories who used the FNLCR protocol would be successful. Participants from eight laboratories saw demonstrations and exchanged information with each other and FNLCR staff.

RAS reference reagents

The RAS Reference Reagents (RRR) Project has the two-fold goal of (1) generating reagents to support the other projects in the FNLCR RAS Initiative, and (2) producing reagents that will assist external RAS scientists with their research. The focus is on the generation of new DNA clones and the development of reagent collections. In FY2016, the RRR program generated over 1,300 individual new DNA clones in support of FNLCR RAS projects and external collaborators. Almost all of the cloning and subcloning was done using Gateway recombinational cloning on our in-house developed combinatorial cloning platform (CCP).

- RAS Initiative support: In total, 833 new constructs were generated in FY2016 in support of RAS Initiative efforts. Ninety-two constructs were produced in support of Project Zero, including constructs for lentiviral delivery of RAS to MEFs and genome engineering constructs using the clustered, regularly interspaced, short palindromic repeat (CRISPR) system. An additional 214 constructs were produced to support assay development, including protein expression constructs for alpha screen development, protein localization constructs for Project 3, and for development of BRET assays. The majority of RRR efforts in support of the RAS Initiative were the production of more than 500 clones for protein expression in support of the biochemistry, biophysics, and structural biology of RAS and RAS effector proteins. This work included the continued development of engineered baculoviruses to permit the facile high-yield production of processed KRAS in insect cells, as well as a number of additional reagents for the generation of high-value structural targets, including NF1, PDE6D, and RAF kinases.
- Support of intramural and external collaborators: More than 100 DNA constructs were generated in support of collaborations with Center for Cancer Research (CCR) investigators, as well as external collaborators and scientists. Among these were 42 clones generated for the laboratory of Dr. Deborah Morrison in CCR for co-development of NanoBRET assays. Additional lentiviral and Escherichia coli (E. coli) clones were made in collaborations with researchers at a number of universities throughout the country.
- RAS clone collections: KRAS entry clone collections were distributed to 17 investigators in FY2016. These samples were sent to diverse locations, including Cornell University, the Dana-Farber Cancer Institute, the University of Liverpool, Baylor University, and the Max Planck Institute in Dortmund.
- KRAS FME production reagents: DNA clones and baculovirus reagents for the production of processed KRAS protein were distributed to nine investigators at nonprofit institutions and were licensed to a company in FY2016. Samples were sent around the world, including to Max Planck, the University of Toronto Medical Center, the Broad Institute, the Fred Hutchinson Cancer Center, and Relay Therapeutics.
- RAS Pathway Collection v2.0: In FY2016, the group finished construction of a 360-clone set of RAS pathway genes cloned in two formats (stop and nostop) in fully validated Gateway entry clones. Clones were sent to the Addgene repository in February 2016, and began distribution worldwide shortly thereafter. As of July 2016, more than 100 clones and five complete sets of reagents have been distributed by Addgene to researchers at more than 60 academic and nonprofit institutions around the world.
- Cancer Toolkit v2.0: In a collaboration with Dr. Kris Wood at Duke University, the RRR group has generated 93 clones based on an improved cloning scheme derived from Dr. Wood's original Cancer Toolkit clone set published in 2015 in *Sci Signal*. The new library uses a new barcoding scheme and better cloning strategies to improve the utility of the clones. This set is in the process of being validated in Dr. Wood's laboratory.

Validation of KRAS as a target

The RAS Target Validation Unit was developed as an additional project/group to (1) pursue the validation of KRAS and/or effectors as targets for therapeutic intervention using novel cell line derivatives, and (2) consolidate and standardize cell line development support to the overall RAS Initiative, in particular the MAPK-dependent RAS-less MEF isogenic cell line panel.

Identification of targets for therapeutic intervention

In collaboration with Dr. Tina Yuan (Broad Institute), this project has been completed using greater than 100 cell lines in 2-D, and a manuscript describing this work has been submitted to *Cell*. The assay (**siR**EN: **siR**NA **E**ffector **N**ode) developed by Dr. Yuan is a multi-parameter flow cytometry–based assay used to measure changes in viability, proliferation, reactive oxygen species, apoptosis, and cell size after node ablation (simultaneous knock-down of a downstream KRAS or a

complete signaling node: e.g., RAF: ARAF, BRAF, CRAF; MEK:MEK1 and MEK2; or ERK:ERK1 and ERK2). The cell lines were selected from the 'GDSC1000' Cell Line Collection (Cyril Benes, Massachusetts General Hospital [MGH]/Harvard), and include 70 mutant and WT KRAS pancreatic and colorectal cell lines analyzed by RAS Initiative staff and an additional 40 lung cell lines that were analyzed by Dr. Yuan. Each cell line has associated gene expression, genomic, and drug sensitivity data that were integrated with the results of the current effort.

- Integrated analysis of gene expression, genomic, and drug sensitivity data with the siREN data was completed by scientists in our group, the RAS Informatics group, MGH, and Selventa.
- Two subtypes emerged from the analysis and were confirmed with additional experiments: a KRAS-subtype and an RSK-subtype, named for the node with the most profound impact on viability outside of the pan-lethal nodes.
- siREN data demonstrated that KRAS-type lines depend on canonical RAS effectors, including RAF and RAC, and upregulate genes involved in the maintenance of the epithelial phenotype. Members of this canonical signaling axis thus represent potential targets for the KRAS-subtype. RSK-type lines express mesenchymal markers and depend on the RSK-MTOR axis to drive oxidative phosphorylation to supplement glycolysis in energy production and/or anabolic metabolism. Many inhibitors of this signaling axis also exist and could be used against RSK-type tumors.
- Based on the results from the integrated analysis, we carried out confirmatory drug screens in a subset of 40 cell lines (21 KRAS-subtype, 19 RSK-subtype) for 28 different compounds using a 10-point dilution series to confirm sensitivity (or resistance) to node perturbation (drug target) predicted by their classification.
- A key genetic determinant of the KRAS/RSK fate is the activation state of STK11 (LKB1). STK11 regulates both metabolism and morphology. Inactivating mutations in STK11 are significantly correlated with the RSK-subtype, and STK11 mRNA levels (inferred biological activity) are strongly correlated with KRAS- and RSK-sensitivity.
- A manuscript has been completed.
- The siREN assay is undergoing modification to extend to 3-D cell culture in a subset of cell lines (pancreas). The goal is to provide a baseline systematic analysis of the cost/benefit to pursuing 3-D cell-based assays as a part of the drug discovery assay portfolio.

MAPK-dependent RAS-less MEF Isogenic Cell Line Panel

The generation, characterization, and validation of an isogenic cell line panel for use in internal and external drug discovery efforts were completed this year. The cell background used for this panel was developed by Dr. Barbacid and adapted for use within the RAS Initiative. The genotypes of the cells are HRAS-/-, NRAS-/-, and KRASfl/fl. The removal of KRAS by Cre recombinase results in G1 arrest, but not cell death in these cells. Proliferation can be rescued by the transduction and expression of a WT RAS, mutant RAS, or MAPK signaling-dependent allele. This cell platform provides a unique opportunity to study aspects of KRAS signaling and biology as a single isoform in an isogenic system. The panel cell lines are now available for free distribution to academic and government laboratories, which have resulted in 15 material transfer agreements (MTAs) since the panel became available in January 2016. Biotech and pharma labs require a licensing agreement to use these cell lines.

- The current panel includes: KRAS 4A WT, KRAS 4B WT, KRAS 4B G12D, G12C, G12V, G13D, Q61L or Q61R, HRAS WT, NRAS WT, and BRAF V600E.
- In support of this effort, more than 500 clones (or subclones) have been generated for downstream characterization. In most cases, a putative clone is not carried forward due to failure to meet validation standards:
  - Confirm clonality using droplet-digital polymerase chain reaction (PCR) and integration analysis (most common reason to abandon further development of a putative clone)
  - Confirm unique clone (based on intergration site profile)
  - Detect stable expression of (RAS) transgene
  - Confirm the loss of endogenous KRAS
  - Detect activation of canonical signaling pathways (MAPK, PI3K, EGFR)
  - Calculate growth rates
  - Exome sequencing to confirm the absence of compensatory mutations in known oncogenes
- There is at least one fully characterized clone per allele available for use in downstream assays.
  - Additional pools have been generated (transduced) and are undergoing cloning and characterization to increase the depth of the panel per allele to account for phenotypes specific to a particular clone
  - Goal is to have at least two lines available per allele

- Cloning and characterization is on-going for the following KRAS mutants to add to the panel:
  - KRAS 4B G12R, G12S
- Pools have been generated for the following KRAS mutants and may be included in the cloning pipeline as projects evolve:
  - KRAS 4B G12A, G13C, A146V, R68S, A146T, S181A, K104A, K104Q, K147L, K147Q, M188L, and K117N

Other 2-D Cell Line Development Support

This group also supported all other RAS Initiative cell line development efforts. End users included the Target Biology-KRAS, Target Biology – Calmodulin, Cryo-EM, and Imaging and Screening Groups.

- More than 150 novel cell line pools generated.
  - Examples: Dox-inducible + fluorescent protein, Dox-inducible + shRNA, overexpression of target gene in human cell line background
  - 200 clones generated from selected pools
- Receives, expands, and generates viable frozen cell line stocks from RAS Initiative collaborators.
- Performs cell line quality control testing: mycoplasma, cell line authentication (short tandem repeat or lentiviral integration analysis).
- Coordinates shipments of (panel) cell lines to requesting investigators.

RAS Program Bioinformatics

The RAS Initiative Informatics Support team provides collaborative scientific support to the RAS Initiative through a layered model including infrastructure support, application development, and data mining and direct data analysis support. Past annual reports have described the rationale of the approach and itemized the components of each layer, as well as how these layers are intended to constitute a seamless integrative environment covering the needs of the RAS Initiative.

The overall efforts under the initiative and the collaborative spokes have progressed through stages of concept testing and research and development to production stage workflows. In response, the Informatics Support group's focus has transitioned with a higher proportion of the effort being directed towards direct data analysis support. This has been primarily manifested as analytical workflow development to facilitate data management, analysis, interpretation, and visualization from these various assays and other research approaches. At the same time, the infrastructure established during the early years, including databases housing many of the publicly available cancer-related genomics resources, has proven to be tremendously useful in the context of adding value to the findings from the research efforts. As such, the continued expansion of these resources, including new database additions and new data mining tools, remains one of the focuses of the group. In addition, we continue to deploy additional mechanisms to make the information in the databases, the assays, and other research findings accessible to the team in ways that they find most informative and intuitive.

Infrastructure

The team has developed a data and experiment-tracking system that is used across the different projects and groups within the initiative. In the last year, the system has continued to evolve with more users, more data types being registered, and much more data incorporated. Currently the RAS labs store protocols, experimental data, and results files in the RAS database, which allows access across groups and allows investigators to cross-references results from different groups. All data associated with the 1,689 cell lines produced in RAS projects are warehoused and available in the database. This includes information on over 15,000 samples, 8,850 images and documents, and 5,000-plus experiment results and procedures. In addition, all protein productions and associated data is stored in the database, accounting for 800-plus full production runs, over 500 Mass Spec runs validating the production proteins, and almost 1,000 experiments recorded in the database with the resulting 22,000 protein samples. In total, the RAS database tracks over 37,000 samples, 9,000 data files and documents, and data for over 10,000 experiments. Lab schedules, product/sample requests, quality control (QC) (and other) analyses, and project tracking are all coordinated in the RAS databases. This is over 200GB of experimental data warehoused and accessible across the RAS projects.

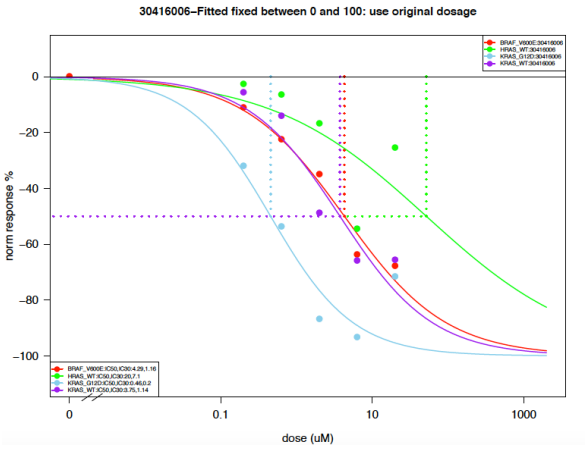
These data are cross-referenced across groups and labs and samples and experiments, allowing investigators to follow samples and results across various investigations. All data is backed up and secured with NIH credentials and easily searched and explored with a user-friendly and flexible interface customized to individual groups' needs. Constant updates and improvements are made to interface with new labs, new interfaces, and new equipment as needed and as projects evolve.

One invaluable aspect of this database system has been monitoring project progress, QC, and managing requests among labs and groups. Samples are all tracked with a barcoding system and samples are, therefore, easily tracked through their production and use in experiments, helping to ensure accuracy and quality of results. Also, requests between groups are handled through the database system for various parts of production. For instance, protein requests are handled through the database system, which ensures needs are met and that the correct proteins are provided with all associated metrics and QC results. This allows results to be obtained and verified quickly and any spurious experimental results investigated fully.

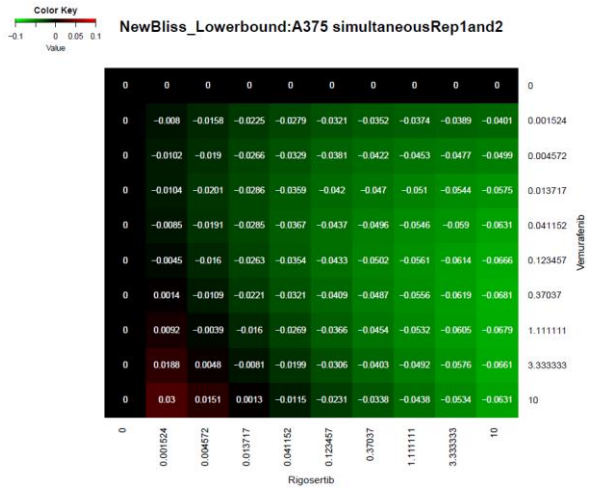
Direct Experimental Analysis Support

The informatics group has provided analysis support for the RAS assay model development group, the Cell and Biochemical Assays Development Group, and the Imaging/Microscopy Group. In each instance, the group has leveraged publicly available software packages to ensure that current methodologies are applied. These packages are then wrapped into a framework that provides automation, integration of results from multiple packages for comparison purposes, and visualization modules to enhance the interpretation of results. As many of the same data analysis methods are applied across different assay methods utilized by the different groups, this provides additional opportunities for code reuse. The main obstacle in these applications is that different applications require different plate layouts and thus there is a considerable requirement for plate-remapping and formatting for analysis. We are working with the lab teams to identify reusable plate layouts to decrease this burden, but the availability of different modules with differing input layouts has also produced a robust palette that covers all layouts identified to date. The analysis workflows can be broken into the following categories:

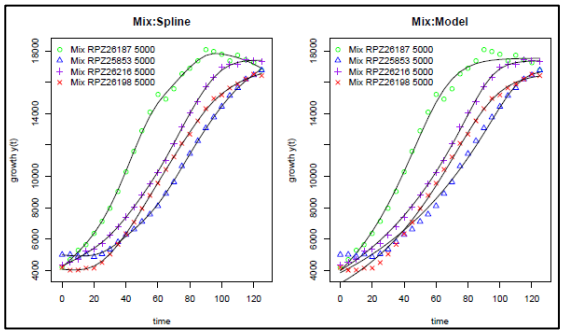
1) Single-drug response curve fitting analysis for the cell and biochemical assay development group for their in-house or NCATS compound screening datasets, the Imaging /Microscopy Group for natural plant extracts and compound screening datasets, and the cell line characterization and development group for their drug response validation datasets for the siREN project (collaboration with Dr. Yuan). Here, a variety of available curve-fitting methods, including the drc R package, are used as a core with extensive modifications to the procedure to allow exhaustive and iterative curve fitting for best results according to collaborators' needs. Once obtained, the fitted data are used to derive IC50, AUC, and other evaluative metrics. As mentioned above, in order to facilitate the analysis and accommodate changing screening plate layouts, different quality assessment metrics, different result visualization schemes, plate-reformatting, and mapping functions were built along the way. These were extended for the SiREN drug data fitting and analysis that has resulted in the SiREN manuscript. The drug curve-fitting graphs were used directly from the workflow outputs.



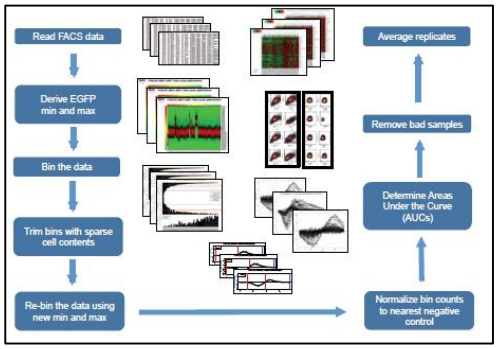
2) Drug combination screening for the cell and biochemical assay development groups (drug synergy and mechanistic evaluation). This relatively new type of data analysis is still evolving data analysis tools and methods. We designed analysis workflows that produce both table and heatmap-based visualizations of this complex data. Methods assessed included: CompuSyn, which is based on the Chou Talalay method, and models including HSA, Bliss, and Loewe as well as the very recently developed new Bliss and ZIP models. For this, we tested a variety of available R packages or functions including Combia, synergyfinder, and the new Bliss model, and also produced in-house R functions for the Bliss and HAS models. These were applied to analysis of the datasets and combined with a series of assessment metrics, results visualization, and plate-reformatting mapping functions in order to accommodate plate format and layout changing schemes to meet the collaborators' needs.



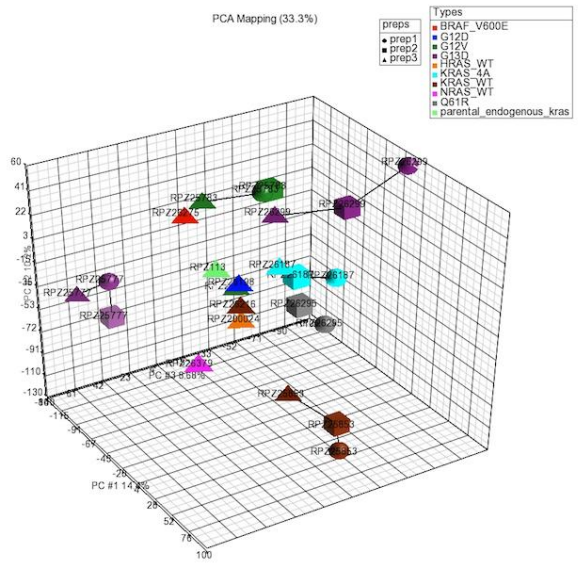
3) In addition to the drug curve fitting, the cell model development group has also applied growth curve analysis to evaluate different cell line models. For this, we evaluated and applied several R packages such as cellGrowth, nlme, and grofit R packages. These were coupled with several customized visualization and data assessment metrics derived outputs.



4) siREN data analysis for the cell line characterization and development group (see above). In addition to the collaboration with Dr. Yuan, we also analyzed data for Dr. Udo Rudloff at NCI with a specific interest in discriminating KRAS G12R mutants from G12V and G12C mutants in pancreatic samples. For this, we built a series of semi-automated scripts to process the siREN data for downstream high-level analysis. We tested and applied a variety of multidimensional analysis methods and strategies to analyze the multi-channel siREN data. These data were integrated with genomic data such as mutation and expression data to further build analytic connectivity with enrichment analysis (Dr. Rudloff). We also applied a global correlation analysis to help uncover the classification groups within the siREN data for the cell lines for underlying biological themes.



5) There are several lines of evidence suggesting that different mutant KRAS alleles, such as G12R and G12C, are biochemically and clinically distinct. If so, then clonal cell lines produced by transgene expression of different alleles in otherwise identical RAS-less MEFs should reveal different gene expression signatures. The cell line model development group and the genomics groups produced RNASeq expression data for these lines, and the informatics group analyzed those data. Principal component analysis (PCA) to assess the data quality and overall behavior of these lines showed that, in fact, the principle genes that discriminate them are not related to the RAS allele, suggesting very minimal allele-specific differences relative to the background genetic differences harbored by each of the clonal cell lines. However, while we were limited by the number of distinct clones for each allele, we did identify differential gene lists that shared common gene-related enrichment terms when comparing the G13D and G12V mutants with their WT counterparts. Thus, there may be very small differences that are only observed in the context of a very uniform isogenic background. Methods applied were limma-voom, edgeR, and DESeq2 followed by pathway enrichment analysis and pathway pattern extraction using an in-house developed PPEP method for biological themes.

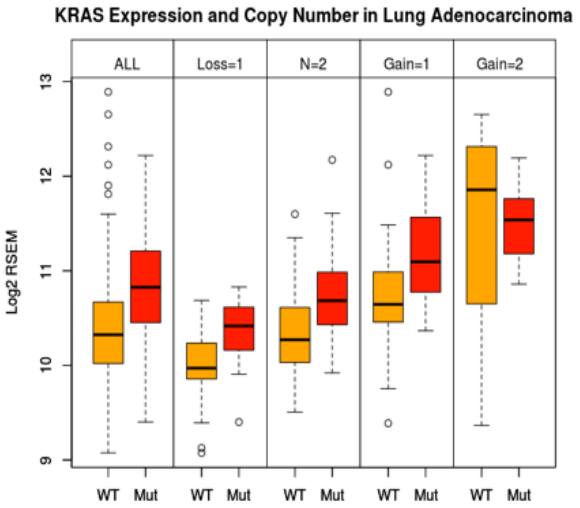


6) One of the projects within the Imaging/Microscopy Group assesses single-molecule tracking of KRAS molecules in live cells. This analysis requires a complex image analysis environment consisting of different open-source applications. We are in the process of reproducing this environment on a server so that the many manual manipulations can be automated. In addition to greatly facilitating a laborious process, this approach will eliminate the possibility of human error in file naming and other steps, dramatically increasing the reliability of the overall analysis.

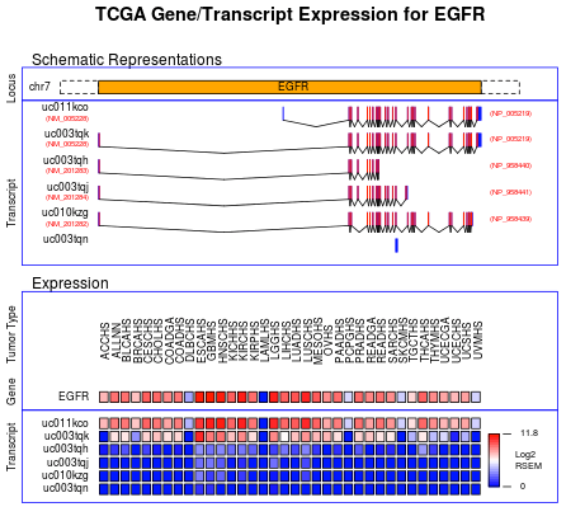
Data Analysis and Mining

We have continued to perform data analysis and mining of publicly available data sources such as TCGA, CCLE, and ICGC with the goal of informing RAS biology. This year, we have completed an analysis examining the link between KRAS gene expression levels and KRAS mutation status. That work, now in the form of a manuscript revealed that part of the increase in expression observed is the result of copy number changes. In the figure below, the different copy number states of KRAS mutant and KRAS WT samples were compared. As can be seen, the expression across all copy number variation (CNV) states is increased in the mutant sample subset relative to their WT counterparts, consistent with the existence of an additional factor (or factors) that further increases expression. In that same study, we revealed associations between the other RAS genes and their own expression in some tumor types and also an

association between the expression levels of RAS genes and the mutational status of the other RAS genes, but also by both upstream (EGFR) and downstream (BRAF) genes in the RAS pathway. The suggestion of expression-level influences of mutant genes on neighboring genes in the RAS pathway has ramifications on both tumor maintenance and the development of resistance that can be further studied.



As part of our effort to facilitate investigator-driven data analysis, we have also begun to implement various web-based data analysis tools. While there are many portals available that provide interfaces to the data from TCGA (e.g., cBIO), we have found that many investigators still do not find these tools to be sufficiently intuitive to navigate or do not directly address their specific questions. We also feel that by providing results more in the context of our very RAS-centric view, we will further benefit our investigators. As an example, one of the many confusing aspects of the RAS pathway is that many of the signaling molecules are highly redundant. For example, there are three RAS genes that have been shown to have highly overlapping functions. However, we do not observe similar frequencies in either mutation or amplification of these genes across different tumor types. Therefore, assessing the expression of the different redundant genes and their different transcriptional products across tumor types may be informative in many ways. To address this question, we have built a web tool that takes a gene symbol as input and then produces a graphic like the one below showing the expression for the whole gene and all of its registered transcripts across tumor types. Of course, we can easily adapt this to produce panels for multiple genes within a specific signaling node or show the comparative expression changes between tumor and normal samples. We anticipate that, as these applications come into use within the Initiative team, we will have many more similar requests.



One of the open questions regarding RAS biology, and specifically KRAS, is the question of the role of KRAS4A. One aspect of this question concerns the actual structure of these two transcripts. The highly referenced Ensembl view of these transcripts, which is frequently used for RNASeq quantitation, shows the two transcripts as having very different 3' UTR regions, with KRAS4A having about a 1.3 kb UTR and KRAS4B having a 4.5 kb UTR. In contrast, then, NCBI Refseq annotations show both transcripts with the longer form of the UTR. We have addressed this question using the PacBio longread technology and have shown that the NCBI model is correct—both KRAS isoforms have the longer form of the UTR. We did identify false termination within this long UTR that can be attributed to polyA tracts within the genomic region from which it is derived.

The funding for the TCGA project is now complete, and while several tumor type–specific analysis working groups continue to analyze these data, there will not be any additional data produced. Still, we expect as additional data produced by the many additional cancer genomic studies underway is combined with this rich resource, many novel approaches to characterize the global changes that are required for the transition from a normal cell to a cancer cell will emerge, and so we continue to analyze the data with new methods. One example would be to use the in-house developed analysis approach to search the TCGA databases for candidate genes in RAS pathways that have significant association with survival outcome through their expression levels either in RAS-involving tumor types or other tumor types, which would potentially help uncover the significance of genes in cancer prognosis and mechanisms as well as biomarkers for outcome.

RAS – Electron Microscopy Laboratory/Center for Molecular Microscopy

The Center for Molecular Microscopy (CMM) is collaborating closely with the RAS program. In FY2016, members of CMM have worked on negative staining and cryo-EM of NF1 domains and RAS-nanodiscs and on the development of cloneable tags that can be used to image in electron microscopy. Such tags will be used to follow the transport and final location of RAS and RAS mutants in cells and study these in 3-D with the FIB-SEM technology.

RAS Optical Microscopy

RAS Microscopy conducts research to understand the mechanisms of RAS activation at the plasma membrane.

The following fluorescence microscopes are available at the ATRF: Nikon NSTORM for super-resolution imaging, a spinning disk confocal microscope, and a laser scanning confocal microscope with fluorescence correlation spectroscopy instrumentation. All three instruments are providing fluorescence microscopy support, including imaging of 96 well plates for identifying compounds that disrupt KRAS.

RAS – Protein Characterization Laboratory

The Protein Characterization Laboratory (PCL) continues to provide the support to the RAS mission on intact protein analysis, post-translational modification detection, and RAS inhibitors analysis. In FY2016, our group has developed intact protein analysis protocols for the quality control on the RAS protein production. We also provide proteomic support on a number of RAS projects. For example, PCL has worked with Dr. Matt Holderfield's group to study RAS modifications and interacting proteins. Using our unique expertise, PCL staff has made significant contributions for RAS projects. As a result, the KRAS mass spectrum produced by PCL staff has been shown on many NCI/CRTP RAS presentations and the publication. PCL staff also developed assay protocols for a number of RAS small molecule inhibitors to quantitatively characterize the affinity and potency of these inhibitors.

PCL provides dedicated mass spectrometry support to the Division of Cancer Epidemiology and Genetics (DCEG) on hormone quantitative analysis. We currently have three full-time equivalent (FTE) employees in the Hormone Analysis Unit (HAU) funded through a Yellow Task. In the past year, HAU/PCL has been doing androgen analysis for about 2,000 WHI-OS samples and estrogen analysis for about 340 samples from BFIT cohort study, as well as 500 samples from the other studies. HAU also co-authored 10 scientific publications with DCEG.

RAS – Protein Expression Laboratory

In addition to cloning support through the RAS reference reagents program, the Protein Expression Laboratory (PEL) supports the RAS program through large-scale protein expression and purification. In FY2016, this group was responsible for purification of over 300 batches of protein (more than seven grams of total protein) for biophysical and structural analysis. This work involved the generation of nearly 500 liters of E. coli expression material and more than 50 liters of insect cell culture. Major accomplishments of the group in FY2016 involved the generation of 15N labeled processed KRAS for NMR spectroscopy, production of subdomains of NF1 (which have previously never been successfully purified), and production of RAF and RASA1 domains utilized in the generation of high-resolution crystal structures of complexes with RAS.

In the final year of a cCRADA with Biogen Idec, PEL collaborated on the production of high-value reagents for early-stage drug development in the Molecular Discovery group at Biogen. Work in FY2016 focused on scale-up production of MLKL1 pseudokinase proteins, early-stage production of phospholipase D1, and screening work on OGA proteins. This work included more than 30 small-scale protein production scouting projects and pilot-scale growths of several hundred milligrams of MLKL1. These proteins have been successfully used by Biogen for biochemical and structural work, as well as in assay development for drug screening.

RAS – Genomics Laboratory

The Genomics Laboratory (GL) has provided support for RAS reference reagent production and cell-line characterization. RAS reference reagent support has been primarily Sanger sequencing of constructs required for the production of plasmid used for the production of proteins and for lentiviral constructs. Cell line support has included gene expression studies utilizing Affymetrix microarrays and RNA-Seq on the Illumina platform. Integration analysis of modified mouse embryonic fibroblast (MEF) cell line has relied on droplet digital polymerase chain reaction (PCR) and on novel strategies for mapping lentiviral integration sites within the cell pools or clones. Whole-exome sequencing has been completed for a large proportion of cell lines that are being used in the internal and external RAS projects. Support has also been provided for the molecular characterization of CRISPR-Cas9 targeted deletion or knocking out or in of specific genetic changes for the RAS project.

The GL is participating in a contractor Collaborative Research and Development Agreement (cCRADA) with the University of Maryland and has established methods for identifying viral infection in formalin-fixed, paraffin-embedded (FFPE) clinical specimens. Known viruses are identified through Next Generation Sequencing on the Ion Torrent Platform. We are now developing bioinformatics tools to identify novel viruses using this technology.

The Clinical Laboratory Imrovement Amendments (CLIA) laboratory within the GL has established new assays for molecular subtyping of lymphomas on the NanoString platform and for the identification of

pharmacogenomics markers from small volumes of blood on the Affymetrix DMET platform. The pharmacogenomics effort supports both CCR investigators and a funded program from the NIH Clinical Center. Molecular Subtyping on the NanoString platform is serving an NCI-sponsored clinical trial and is supported through a Technical Services Agreement (TSA) with an external partner.

National Cryo-EM Facility (NCEF)

The National Cancer Institute, NIH has committed to establishing a national cancer research community-accessible Cryo-EM user facility that will be managed and operated by FNLCR. The purpose of the facility is to enable the collection of high-quality cryo-EM images, with minimal delay between request for access and data collection. It is expected that NCEF will be ready to accept users in the fall of 2016. Specialists in cryo-EM data collection and microscope operation will staff the facility and assist users with data collection. The facility will include an initial Titan Krios microscope, and additional microscopes may be added in future years.

This pilot facility will begin to address the pressing national and regional need that U.S. structural biologists have access to advanced microscopes capable of supporting high resolution Cryo-EM studies. Cancer-related projects, especially those analyzing the macromolecular complexes that drive oncogenesis, will have priority. The facility will provide rapid access on a first-come, first-serve basis for a nominal cost to qualified users that can provide evidence that they have pre-screened grids ready for high-resolution data collection.

The CRTP will host and help establish the NCEF. NCEF will exist alongside other CRTP/FNLCR entities that support the NCI Mission (RAS Initiative, NCL, ACL) and CCR-dedicated support labs such as the CCR Center for Molecular Microscopy, which carries out cryo-EM for intramural investigators.

NCEF is being established under the authority of the FNLAC and will be funded directly from the office of the NCI Director. Dr. Sriram Subramaniam (NCI) will provide technical and scientific oversight for the NCEF, and the NCI contracting officer's representative (COR) is Dr. Sara Hook.

Partnership Development Office

The Partnership Development Office (PDO) oversees and manages all CRADA opportunities for the Frederick National Laboratory for Cancer Research (FNLCR), which includes contractor CRADAs (cCRADA) and Technical Services. Management of the cCRADA portfolio is under the purview of the latest hire for the PDO, Dr. Vladimir Popov. Dr. Popov is trained as a cancer biologist and was a fellow at the Penn Center for Innovation and the University of Pennsylvania, and he became a Technology Licensing Specialist there before joining FNLCR. This year, there were six cCRADAs completed with NCI-designated cancer centers, large pharmaceutical companies, and small biotechnology companies. There are currently an additional 12 opportunities under review. The total revenue to FNLCR is approximately $600,000. However, the total value to the program is significantly higher when examining the scientific impact of these projects to the broader cancer and HIV communities. In additional to the cCRADAs, there were two materials cCRADAs executed. These are important, strategic agreements that have allowed FNLCR investigators in the Aids and Cancer Virus Program (ACVP) to have access to the most current anti-retroviral drugs that are critical to carrying out their research. The technical service program saw one new service added to the portfolio that emerged as an important new Clinical Laboratory Improvement Amendments (CLIA) diagnostic. There were 55 agreements and amendments executed over the past year with a total value of approximately $1.2 million.

NCI at Frederick Office of Scientific Operations

Support Provided by the AIDS and Cancer Virus Program

Research Support Cores

Quantitative Molecular Diagnostics Core

The Quantitative Molecular Diagnostics Core (QMDC) provides state-of-the-art quantitative molecular analyses to measure specific nucleic acid sequences in provided specimens, meeting evolving changes in demand for targets, samples, and analyses to support studies within the AIDS and Cancer Virus Program (ACVP), intramural National Institutes of Health (NIH) laboratories, and in the extramural community. The QMDC performs testing for simian immunodeficiency virus (SIV) and related viruses, determining viral loads in specimens from nonhuman primate (NHP) models for AIDS, including plasma viral loads, cell-associated viral loads, and tissue-associated viral loads.

For FY2016, at the current level of throughput, the QMDC will have handled more than 15,000 plasma samples and more than 1,000 isolated cell samples and will have assayed approximately 6,000 tissue and biopsy samples using ultrasensitive, hybrid real-time/digital nested polymerase chain reaction (PCR) assay formats. This assay throughput included a significant fraction of demanding, time-critical, rapid-turnaround assays in support of repeat challenge vaccine studies. In FY2016, in addition to both developing new droplet digital PCR assays for SIV nucleic acids and supporting ACVP internal studies, the QMDC provided key enabling collaborative support to multiple high-profile studies by extramural investigators addressing questions such as

viral spread after mucosal inoculation (Barouch DH et al., *Cell*, 2016, 165:656), durability of antibody-mediated passive immunoprophylaxis (Gautam et al., *Nature*, 533:105, 2016, 11:e1004633), development of improved SHIVs (Li et al., *Proc Natl Acad Sci USA*, 2016, 113:E3413-22) and antiretroviral treatment responses in naturally infected sooty mangabeys (Calascibetta F et al., *J Virol*, pii: JVI.00598-16. [Epub ahead of print]), with the final application requiring de novo development of a novel, customized assay to address the viral sequence diversity found in naturally infected animals.

HIV Molecular Monitoring Core

Established in FY2011, the HIV Molecular Monitoring Core (HMMC) performs specialty services including ultrasensitive HIV-1 viral load measurements from plasma, CSF, cells, and tissues, as well as single-genome sequencing and analysis. The HMMC originated in response to investigator requests for continued and expanded access to these specialty services, initially developed by the HIV-Drug Resistance Program's (DRP; Center for Cancer Research (CCR)/NCI) Virology Core. Services are provided for specimens from qualified, government-supported studies, allowing the HIV-DRP Virology Core to focus on developing alternative assays, potentially for transfer to the HMMC as appropriate, and on research support services for the DRP investigators. The quantitation activities and operations of the HMMC are located in Building 432, while the single-genome sequencing activities of the HMMC are resident with the Viral Evolution Core (VEC) of the ACVP in Building 535, under the direction of Dr. Brandon Keele.

The HMMC's quantitation group utilizes its established multiplexed, hybrid real-time/digital assay format, which interrogates multiple sequence regions in HIV-1 DNA or RNA from nucleic acids isolated from plasma, CSF, cell, or tissue samples. The current assay has improved sensitivity and clade coverage over that transferred from the HIV-DRP, addressing the evolving needs of the research community and expanding the services offered. The HMMC gag Taqman-based assay detects 96% of a panel of 76 patient isolates comprised of clades A, B, C, D, E, F, and G (NIH AIDS Reagent Program) in comparison with the commercial Roche COBAS Taqman clinical assay (within 10-fold of COBAS results; greater than 25,000 copies assayed). The HMMC quantitation group has successfully assayed more than 600 plasma samples, 100 CSF samples, 100 cell samples, and 26 tissue samples this past year. This includes a recently completed 100 plasma qualification panel from the HIV Reservoir Assay Validation and Evaluation Network (RAVEN), Blood Systems Research Institute. Current collaborators include Frank Maldarelli and Bob Yarchoan (CCR, NCI, NIH), Ron Swanstrom and Dave Margolis (UNC), Eileen Scully (Harvard), John Mascola (VRC, NAIAD, NIH), Nina Bhardwaj (MT Sinai), Serena Spudich (Yale), and Irini Sereti (NIAID, NIH). Future collaborative studies include those from Tom Uldrick (CCR, NCI, NIH), Kiat Ruxrungtham (HIV-NAT, Thai Red Cross AIDS Research Center), Marty Markowitz (ADARC, Rockefeller U), Howard Grossman (NYU), Avindra Nath (NINDS, NIH), and Steven Douglas (University of Pennsylvania, CHOP). Publications resulting from the HMMC's quantitation group during the current reporting period include Elliott et al. (*Lancet HIV*, 2015, 2:e520), Lynch et al. (*Sci Transl Med*, 2015, 7:319ra206), and Simonetti et al. (*Proc Natl Acad Sci USA*, 2016, 113:1883).

Another key service of the HMMC is single-genome sequencing and viral evolution. The genomics group has successfully initiated five collaborative studies with intramural and extramural investigators utilizing novel sequencing approaches along with our expertise in viral evolution to better understand viral reservoirs and recrudescent viremia and viral recombination. Our viral recombination studies are in collaboration with Drs. Wei-Shau Hu and Vinay Pathak and colleagues in the HIV Dynamics and Replication Program. In one study, we determined that there was a high level of recombination potential within the HIV-1 clade A virus (Nikolaitchik et al., *Virology*, 2015, 484:334). These data are particularly important since intravenous drug use drives the HIV-1 clade A epidemic in the former Soviet Union with a high potential for infection with multiple variants and recombination. We also completed a second recombination study with these same collaborators in which we determined that G-to-A hypermutation has a minimal impact on HIV-1 recombination and overall genetic variation (Delviks-Frankenberry et al., *PLoS Path*, 2016, 12:e1005646). The genomics group contributed to three collaborative studies for HIV Cure research. With Drs. Jonathan Li (Harvard), Mary Kearney and John Coffin (NCI, DRP), and Dr. John Mellors (University of Pittsburgh), we were able to determine that the origins of rebound plasma viremia included cells that were transcriptionally active prior to therapeutic interruption (Kearney et al., *J Virol*, 2016, 90:1369). We were also able to contribute to a study with Dr. Frank Maldarelli (NCI, DRP) demonstrating that clonally expanded CD4+ T cells can produce infectious viruses and may represent an important source of rebound virus (Simonetti et al., *Proc Natl Acad Sci*, 2016, 113:1883). Finally, the genomics group of the HMMC contributed to a study with Dr. John Mellors (University of Pittsburgh) determining that infectious virus recovery assays underestimate the true latent viral reservoir size (Sobolewski et al., in preparation). Overall, we have leveraged our expertise in sequence analysis and viral evolution to make significant contributions to HIV-1 research in cure and viral recombination.

Biological Products Core

ACVP capabilities include the production and provision by the Biological Products Core (BPC) of antigen capture immunoassay reagents for determination of HIV virus levels in samples from in vitro experiments. During the current year, the BPC provided 930 sets of key reagents required to perform HIV-1 p24 antigen capture

immunoassays to 19 investigators under MTAs. Each reagent set can be used to determine the concentration of HIV-1 p24 in 80 samples. Included in each reagent set is purified capture monoclonal antibody against HIV-1 p24, HIV-1 p24 standard, and the primary anti-HIV-1 polyclonal antibody. These reagents, all produced by the BPC, allow recipient laboratories to set up their own kits using the simple instructions provided. These reagent sets, provided to the research community at no cost, have an estimated value of $279,000.

The BPC has extensive expertise and experience in the production of purified retrovirus preparations at multiple scales and provides these preparations in either infectious or chemically inactivated form. The BPC provided over 265 mg of highly purified and concentrated retrovirus preparations and related control reagents to 23 qualified requesting intramural and extramural laboratories. This service represents an estimated cost savings to the requesting investigators of approximately $155,000, compared to a conservative estimate based on $1,000/ml for commercially sourced purified virus; however, all of the materials provided by BPC were unique reagents that are not available from commercial sources at any cost. In keeping with the mission of a National Laboratory, over 98 percent of this material was provided to extramural investigators. Another key function of the BPC is the small-scale purification of HIV or SIV from new, productively infected cell lines/cell clones under development or from samples submitted by other laboratories for analysis to assess the quantity and quality of the virus in these samples. The aim is either to identify the best cell lines/cell clones for use in future large-scale virus production/purification projects or to assess some compositional element of the virus. Depending on planned analyses, the virus was sometimes chemically inactivated prior to purification. During this period, over 162 preparations were made from nearly five liters of cell culture supernatant. All were analyzed by the Retroviral Protein Chemistry Core (RPCC) (Dr. Elena Chertova, Head) and selected ones were further analyzed by Cryo-EM (Dr. Sriram Subramaniam, NCI).

The BPC has broad experience preparing limiting dilution clones that are productively infected with retroviruses. Productively infected, non-clonal cell lines are typically used to produce retroviruses and consist of numerous independently infected cells that, due to RT error during reverse transcription, produce a “swarm” of genetically diverse progeny virions. The virus yield and genetic distribution in these cultures can evolve, making it essentially impossible to derive compositionally equivalent virus preparations from them over time. Within the limits of somatic cell mutation, clonal cell lines productively infected with retroviruses can consistently produce genetically identical progeny viruses in very consistent yield, making these clones extremely valuable to the research community, which is increasingly in need of isogenic virus preparations for their experiments. The derivation and screening of these clones is laborious and not possible without the extensive expertise provided by the Cellular Immunity Core and RPCC of the ACVP for FACS and Biochemical analysis, respectively. Some key clones prepared include: HIV-1 CH050s TF/A66-R5 clones 1 and 6, which were prepared as part of a collaboration with Dr. Bart Haynes (Duke University), and purified virus preparations from one of these is being considered for use in an experimental HIV-1 vaccine experiment aimed at illuminating a pathway to elicit broadly neutralizing antibodies. HIV-1 LAI BG505.T332N/A66-R5 clones 2 and 4 were prepared as part of a collaboration with Dr. John Moore (Cornell) with the aim of having a source of isogenic HIV-1 particles populated with the BG505 SU protein for structural analysis by cryo-EM in situ and glycan analysis of purified SU protein from virions, both for comparison with the data from recombinant BG505 SU protein that was previously published. A panel of 17 limiting dilution clones of our SIVmac239*/SUPT1-CCR5 CL.30 cell line was prepared with the aim of deriving a source of SIVmac239* virions with enhanced SU protein content. Although the analysis is not complete, it is clear that these clones produce progeny virions with differing SU protein to CA protein ratios, and some appear to have better SU protein content than virions derived from the parental culture.

Cellular Immunity Core

The Cellular Immunity Core (CIC) provides ACVP investigators and collaborators with quantitative multi-parametric cellular analysis and cell separation using advanced flow cytometry methods and instrumentation. Given its infrastructure and enhanced safety protocols, the CIC is the only Frederick National Lab group approved by the Institutional Biosafety Committee to perform fluorescence-activated cell sorting of infectious specimens. In addition, the CIC devises and applies polychromatic flow cytometry methods to monitor phenotypic and functional immune changes associated with disease, clinical parameters, and other study events, in support of internal and collaborative NHP studies.

In utilizing its state-of-the-art resources and technical expertise, the CIC has made many important contributions to HIV, AIDS, and NHP research over the last year by providing immune response monitoring, subset phenotyping, cell sorting and separation analysis, novel assay and reagent development for NHP studies, technical assistance, flow cytometry and immunology consultation and training, and assay and instrument troubleshooting and multiparameter data analysis for many laboratories. Within the ACVP, the CIC provided support for the RPS (Lifson, Schneider, Del Prete), TAC/RIPS (Estes, Deleage), VEC/RES (Keele, O’Brien, Camus), BPC (Bess, Schaden-Ireland, Smith), VOS (Whitby, Rashon, Labo), SSC (Del Prete, Coalter), and RCIS (Ott, Coren, Barsov, Trivett). Supported intramural laboratories include the FNLCR Laboratory of Experimental Immunology (Carrington, Apps), Center for Advanced Preclinical Research (Vilimas), Clinical Services Program

(Krymskaya), Frederick Flow Cytometry Core (Noer), Sequencing Facility (Raley), Cancer and CCR Developmental Biology Laboratory (Mackem, Trofka), Laboratory of Genome Integrity (Livak), and HIVDRP Viral Mutation (Pathak) and Viral Recombination (Hu) Sections. Supported extramural laboratories include the University of Minnesota (Schacker), Wisconsin National Primate Research Center (Smedley), University of Washington (Gale, Davis), Aaron Diamond AIDS Research Center (Hatziioannou, Bieniasz), and USAMRIID Center for Genome Sciences (Kulscar, Sanchez-Lockhart, Hill). The CIC has also provided vital data for multiple publications and scientific conference presentations, including Del Prete, G. et al. *Antimicrob Agents Chemother*, 2015 and Apps, R. et al., *Cell Host Microbe*, 2016.

Over the last year, the CIC has evaluated and developed essential reagents and immunological assays to further our understanding of NHP systems and HIV/SIV research endeavors, and provided support to fulfill ACVP and collaborative project research goals. Some key examples include developing and optimizing polychromatic antibody panels and flow assays to detect macrophage and granulocyte subpopulations in NHP whole blood and tissue samples and assessing their corresponding activation status. Most of these subpopulations have never been fully identified or described in NHP systems. Use of these new antibody panels and flow assays allows us to further define NHP macrophage and granulocyte phenotypes as well as changes associated with SIV infection and treatment. The CIC has also continued to develop, optimize, and assess our flow cytometry–based RNA detection assay as a means to concurrently detect and monitor viral RNA transcripts and surface/intracellular markers at the single-cell level. The CIC has also performed NHP cross-reactivity analysis on several antibodies directed against human surface and intracellular proteins in conjunction with laboratories specializing in human research, other NHP laboratories specializing in NHP models for research, and companies mutually interested in determining the applicability of reagents for use in NHP studies, and this information has been provided to the HIV research community.

In support of (14) NHP studies and a plethora of investigator projects occurring during the review period, the CIC analyzed the following samples, obtained from whole NHP blood, peripheral blood, lymph node, gut- and rectal-associated lymph node tissue, ascites, cerebral spinal fluid and bronchiolar lavage cells, and cell lines: 4,690 samples in 4-8 color absolute cell counting assays; 17,200 samples in 6-16 color phenotyping and immune function assays; and sorted/separated 120 samples.

Retroviral Protein Chemistry Core

The RPCC provides protein chemistry support to the ACVP and collaborating investigators by analyzing purified virus preparations and providing expertise in the purification and characterization of proteins, including recombinant proteins.

The RPCC continues to characterize purified preparations of retroviruses of interest generated either within ACVP or by our collaborators, with analyses that include assessment of the state and amount of surface (SU) and transmembrane (TM) envelope glycoproteins and gag proteins; application of a spectrum of methods, including gel-based calibrated fluorescent staining analysis and immunoblot analysis; and HPLC fractionation and quantitative amino acid analysis and mass spectroscopy, as well as more intensive interventional analyses involving enzymatic digestions or covalent modifications. Different preparations (viral samples for analysis, cell lysate samples, plus samples of monkey sera) were analyzed during the period; 140 gels and 70 immunoblots (most with multiple re-probes) were performed with densitometric and other analyses when appropriate. The majority of this material was provided by extramural investigators. Over the same time period, the RPCC performed quantitative amino acid analysis on 75 samples (both for ACVP and external requests).

RPCC monitored antiretroviral drug levels from in vivo samples from treated SIV-infected macaques, including in collaboration with Dr. J. Balzarini (Rega Institute for Medical Research Laboratory of Virology and Chemotherapy, Leuven, Belgium) for determination of the levels of pradimicin S (PRMS). Based on our data, a regimen was adjusted and the drug was applied only once daily to the monkeys (instead of the anticipated twice daily), improving study conduct and reducing animal stress. RPCC also helped J. Lifson (ACVP) in collaboration with Koen KA Van Rompay (California National Primate Research Center, UC Davis, USA) and Mark A. Wainberg (McGill University AIDS Centre, Lady Davis Institute for Medical Research, Montreal, Canada), to monitor plasma Dolutegravir (DTG) in SIV-infected macaques in a unique study of drug resistance mutations arising in the setting of DTG monotherapy.

A combined glycomics and glycoproteomics analysis was performed for the site-specific analysis of N-linked glycosylation heterogeneity from HPLC-purified HIV-1 BAL/SupT1-R5 gp120 in collaboration with Desrosiers R. (University of Miami Miller School of Medicine, Miami, FL) and A. Dell (Division of Molecular Biosciences, Imperial College, London). Knowing the site-specific glycosylation of gp120 is an essential pre-requisite for the rational design of glycopeptide antigens for HIV vaccine development. The first systematic glycosylation site analysis of a gp120 derived from virions produced by infected T lymphoid cells was performed, and it was shown that a single site is exclusively substituted with complex glycans.

The RPCC, in collaboration with J. Bess (BPC), purified the simian form of IL-7 as a biologically active protein from a supernatant provided by the George Pavlakis laboratory (CCR/NCI) using Capto Q anion exchange chromatography, and further IL-7 protein by reverse-phase HPLC to a high degree of purity. Approximately 26 mg of biologically active simian IL-7 was provided for use in the continuation of in vivo macaque studies.

Viral Evolution Core

The mission of the VEC is to provide expertise in specialized sequencing techniques, molecular cloning, and viral evolution analyses to support the ACVP, NCI, NIH, and extramural investigators. The VEC seeks to increase the overall understanding of viral evolution with a major focus on exploiting the unique advantages afforded by utilizing SIV infection in NHP models. The VEC has established critical infrastructure and essential personnel providing key genetic insights of viral evolution to a diverse group of scientists that might lack the capability and expertise to perform such analyses in their individual laboratories. The VEC supports a number of diverse scientific research objectives that can be categorized into four research areas: (i) preclinical vaccine, (ii) viral transmission, (iii) NHP model development, and (iv) general viral evolution studies.

The VEC spends considerable time and effort in support of collaborative preclinical NHP vaccine studies. This year, the VEC contributed sequencing and analysis in support of five collaborative vaccine studies. Continuing our long-standing collaboration with Dr. Franchini (NCI, VB), we provided transmitted/founder variant analysis as a surrogate for vaccine efficacy to assess immune responses to an ALVAC vaccine comparing alum to MF59 (Vaccari et al., *Nature Medicine*, 2016). Here, vaccine efficacy was associated with alum-induced immunity but not MF59-induced immunity. These data might help inform a pilot HVTN trial testing this exact approach (ALVAC prime and MF59 protein boost) in South Africa (HVTN100). This study was followed up with a collaborative study with Dr. Don Forthal (University of California Irvine) where we found that capture antibodies induced during vaccination were correlated with the number of transmitted/founder variants, suggesting that capture without neutralization was deleterious to vaccinated animals (Gach et al., *J Virol*, 2016, in press). In a vaccine study with Dr. Dan Barouch (Harvard), the VEC was able to show that Adenovirus prime with Env protein boost is able to protect against neutralization-sensitive and resistant SIVsmE660 variants (Keele et al., in review). This study was made possible by a detailed sieve analysis of transmitted/founder variants and a genetic analysis of neutralization resistance and sensitivity. This is the first study to show that protection from acquisition can be achieved with a neutralization-resistant form of SIVsmE660. Finally, in collaboration with Drs. Cristian Apetrei and Ivona Pandrea (University of Pittsburgh), we showed that Maraviroc does not prevent oral transmission of SIV in infant macaques (Brocca-Cofano et al., in review).

To better understand viral transmission, we developed a rhesus macaque model to identify the routes and dynamics of systemic dissemination of SIV following mucosal transmission. Rhesus macaques were intra-vaginally challenged using SIVmac239X, a synthetic swarm virus genetically tagged for variant enumeration consisting of a mixture of 10 individual viral lineages. The proportion of each variant within the inoculum was determined using a novel real-time single-genome amplification assay. Rhesus macaques were sacrificed at different early time points post-challenge (days three through 14) and individual viral linages were tracked in 17 different tissues and blood using SGA and next-generation sequencing. We showed that vaginal mucosal infections are highly variable in the number of variants initiating infection, from one to 10 variants. We also observed different profiles of dissemination from local sites of exposure to systemic compartment with some animals presenting a rapid systemic dissemination, while other were found only at local sites with no evidence of dissemination. This observation was independent of the duration of infection. We further observed a significant recruitment of CD4+ T cells and myeloid cells at the site of infection and an increased expression of different host restriction factors; however, these factors were found in both sites of active replication and sites without detectable virus. Insights from the sequencing analysis provided by the VEC highlight the unique utility of the molecularly tagged virus model.

To support novel NHP model development, the VEC has continued its long-term evolutionary analysis of a minimally chimeric HIV/SIV infection in pigtail macaques with Drs. Jeff Lifson (ACVP), Vineet KewalRamani (NCI), and Theodora Hatziioannou and Paul Bieniasz (Aaron Diamond Research Center). Here we sequenced both minimally chimeric simian tropic HIV (stHIV) and HIV-Env based SHIVs to determine in vivo adaptation and viral selection in various NHPs. We also use sequencing to determine the most fit viruses when animals are challenged with a mixture of different viruses in a single pool. This approach allows for the assessment of many different viruses within the same animal, saving resources and reducing the number of macaques needed to test new SHIV clones. We have successfully identified several useful SHIV clones using this model.

The VEC also collaborated with several internal and extramural investigators on several interesting studies requiring analysis of viral evolution in various host species with unique virus populations. In one study with Drs. James Hoxie (University of Pennsylvania) and Andrew Lackner (Tulane), we demonstrated that elimination of a signaling motif in gp41 of the envelope gene allows for virus control, CD4+ T cell sparing, and enhanced mucosal immunity (Breed et al., *J Virol*, 2015). The VEC provided sequence analysis of how the virus

evolved in some animals to gain back some of the functions of this diminished virus. In a study with Dr. Marjorie Robert-Guroff (NCI, VB), the VEC provided sequence analysis of viruses that had been sorted using flow cytometry (Musich et al., in preparation). Lastly, we were able to provide sequencing support to the QMDC in establishing a real-time, quantitative PCR assay to quantify sequence divergent SIVsm variants in naturally infected sooty mangabeys. This assay was used to assess the impact on SIV replication of combination antiviral therapy (cART) in this host species in which SIV infection is typically nonprogressive (Calascibetta et al., *J Virol*, 2016). cART-suppressed plasma viremia and rebound viremia was rapid following removal of therapy, with specific immunological changes occurring during therapeutic suppression.

Tissue Analysis Core

The Tissue Analysis Core (TAC) utilizes state-of-the-art tissue-based methods; combining immuno-histochemistry (IHC), immunofluorescence analysis (IFA), in situ hybridization (ISH), and laser capture microdissection (LCM) to better understand HIV/SIV mucosal transmission, pathogenesis, and therapeutic intervention strategies. Working with the RIPS, the TAC developed and validated a new next-generation ISH approach that, for the first time, allows for the reliable robust detection of vDNA in tissue sections, as well as a multiplexing capability to detect vRNA and vDNA on the same tissue section (*Pathog Immun*, 2016, 1: 68-106). This new approach provides a powerful tool to identify and characterize viral reservoirs in situ. The TAC supports numerous collaborative ACVP, intramural, and extramural scientific research projects aimed at better understanding HIV/SIV mucosal transmission, pathogenesis, reservoir establishment and persistence, and the development of novel lentiviral animal models. During FY2016, the TAC continued to handle, process, and perform IHC, IFA, ISH, and LCM experiments on tissue samples in support of all ACVP NHP studies as well as numerous collaborations with both intramural and extramural scientists that have resulted in 12 publications during this period in high-tier journals (i.e., *Antimicrob Agents Chemother, Blood, Curr HIV/AIDS Rep, J Clin Invest, J Immuno, J Infect Dis., J Virol, Mucosal Immunol, PLoS Path*, and *Proc Natl Acad Sci*). Furthermore, during FY2016, the TAC executed/completed three large TSAs (Brigham and Women's Hospital [ACTG], Fred Hutchinson Cancer Research Center [UW] and Emory University), and completed one cCRADA with the University of Minnesota.

TAC accomplishments for FY2016 include key contributions to ACVP, intramural, and extramural studies. Highlights of TAC work include contributions to the following studies: (1) The generation and characterization of pathogenic transmitted/founder molecular clones from SIVsmE660 and SIVmac251 following mucosal infection (J Virol, 2016 Jul 13. pii: JVI.00718-16). (2) Demonstrating that IL-15 promotes activation and expansion of CD8+ T cells in HIV-1 infection (*J Clin Invest*, 2016 Jul 1;126(7):2745-56). (3) The characterization that envelope residue 375 substitutions in SHIV enhances CD4 binding and replication in rhesus macaques (*Proc Natl Acad Sci USA*, 2016 Jun 14;113(24):E3413-22). (4) Ascertaining that MRSA infections in HIV-infected people are associated with decreased MRSA-specific Th1 immunity (*PLoS Path*, 2016 Apr 19;12(4):e1005580). (5) Determining that enhancement of microbiota in healthy macaques results in beneficial modulation of mucosal and systemic immune function (*J Immuno*, 2016 Mar 1;196(5):2401-9). (6) Showing that IL-21 and probiotic therapy improved Th17 frequencies, microbial translocation, and the microbiome in ARV-treated SIV-infected macaques (*Mucosal Immunol*, 2016 Mar;9(2):458-67). (7) Determining that administration of interleukin-7 increases CD4+ T cells in idiopathic CD4 lymphocytopenia (*Blood*, 2016 Feb 25;127(8):977-88). (8) Determining that Romidepsin-Treatment results in elevated plasma viral loads in SIV-infected rhesus macaques on suppressive cART (*Antimicrob Agents Chemother*, 2015 Dec 28;60(3):1560-72). (9) Determining that interleukin-21 combined with ART reduces inflammation and viral reservoir in SIV-infected macaques (*J Clin Invest*, 2015 Dec;125(12):4497-513). (10) Showing that patients with ICL, despite gut mucosal lymphopenia and local tissue inflammation, have preserved enterocyte turnover and T-helper type 17 cells with minimal systemic inflammation (*J Infect Dis*, 2015 Nov 15;212(10):1579-87). (11) Determining the differential impact of in vivo CD8+ T lymphocyte depletion in controller versus progressor SIV-infected macaques (J Virol, 2015 Sep;89(17):8677-86). And (12) Showing that dysbiotic bacteria translocate in progressive SIV infection (Mucosal Immunol, 2015 Sep;8(5):1009-20).

Specimen Support Core

The Specimen Support Core (SSC) enables ACVP investigators and their collaborators to conduct in vivo NHP studies of AIDS pathogenesis, treatments, and vaccines by providing immediate upstream and downstream support for all protocol-associated treatments and specimen collections. Specific functions include: i) coordinating and scheduling all ACVP NHP study events to meet scientific study objectives, ii) preparing appropriate specimen collection containers and arranging specimen transport, iii) receiving and processing whole blood and tissue-derived specimens from macaques on study to component samples specifically prepared for an assortment of assays, iv) accessioning, distributing, and storing generated specimens, v) maintaining detailed databases of these specimens, including inventories of cryo-preserved samples, and vi) formulating and preparing unit dose forms of anti-viral drugs and other compounds for administration to study animals.

In FY2016, the SSC provided infrastructure support for nine unique NHP animal study protocols conducted by ACVP investigators. This support involved the processing of approximately 39,000 ml of whole blood, more than 230 lymph node tissue specimens, more than 340 intestinal tissue samples, and more than 20 bronchoalveolar lavage samples for studies conducted by ACVP and collaborating investigators. This resulted in the generation, cryopreservation, and tracking with retrieval on demand for analysis of approximately 17,000 vials of plasma, more than 12,000 vials of viably cryopreserved PBMC, and more than 9,000 vials of frozen cell pellets, as well as the production of over 4,200 fresh mononuclear cell and whole-blood specimens for flow cytometric analyses. The SSC also provided initial processing assistance for more than 2,900 fixed-tissue specimens. In FY2016, the SSC additionally prepared more than 1,300 single-dose vials of powdered drugs and 1,600 ml for oral administration to animals on study, and formulated over 1,600 ml of liquid drug preparations aliquoted into more than 400 vials for administration by injection. Two new post-doctoral fellows in the program, Drs. Adrienne Swanstrom and Celine Camus, received laboratory training on blood and tissue processing techniques by SSC staff. SSC personnel subsequently worked closely with Dr. Camus to develop and evaluate a lymph node processing method to allow for improved isolation of follicular dendritic (fDC), and this technique has since been applied by the SSC to provide fDC enriched single-cell suspensions from lymph node tissue upon request.

ACVP Principal Investigator Research Sections

Retrovirus-Cell Interaction Section

The goal of the Retrovirus-Cell Interaction Section (RCIS) is to study the interactions between HIV-1/SIV and their host cells to address important biological questions, emphasizing T-cell-mediated antiviral immunity and mechanisms for T-cell resistance to HIV/SIV infection.

Significant Achievement

Preferential targeting of CD8+ T cells to B-cell follicles by CXCR5 transduction. The RCIS continued work focused on engineering CD8+ T cells with the CXCR5 homing receptor to redirect them into B-cell follicles in rhesus macaques. Normally, CD8+ T cells, including those that are effective against HIV-1/SIV, are essentially excluded from B-cell follicles. In AIDS virus-infected individuals, B-cell follicles also harbor significant levels of infected CD4+ T cells. Since a primary mechanism for AIDS virus control is clearance of infected cells by cytotoxic CD8+ T cells, B-cell follicles present a sanctuary for virus infected CD4+ T cells to escape the action of antiviral CD8+ T cells. Indeed, even in HIV or SIV infected individuals able to spontaneously control viral replication by virtue of particularly effective CD8+ T-cell responses restricted by protective MHC-I alleles ("elite controllers"), infected cells are readily detectable within B-cell follicles. In infected non-controllers on cART, infected cells are progressively restricted to B-cell follicles but become more widely distributed after cessation of treatment. Thus, placing effective antiviral CD8+ T cells into this viral sanctuary could reduce this contribution to persistent low level viremia and viral recrudescence. Building on our initial successful demonstration of enhancing trafficking of adoptively transferred, molecularly engineered CD8+ T cells into B-cell follicles by CXCR5 transduction, the RCIS has been developing approaches to produce SIV-specific CD8+ T cells co-expressing both an SIV-specific TCR capable of strong virus suppression and CXCR5 to provide an effective antiviral response inside follicles. Working with this new CXCR5-mediated CD8+ T cell targeting system, the RCIS produced and adoptively transferred large numbers of SIV-specific TCR/CXCR5-engineered T cells in rhesus macaques, and in a pilot study with the Retroviral Immunopathology Section, demonstrated that the infused cells preferentially trafficked to B-cell follicles in spleen and lymph nodes, with improved persistence. Current work focuses on applying lessons learned from these successes to larger studies in rhesus macaques to test whether placing an effective antiviral response inside B-cell follicles can suppress the residual virus hiding in these sanctuaries.

Suppression of AIDS viruses by antiviral CD4+ T cells. The RCIS published a study, (Ayala et al., *Virology*, 2016) examining SIV infection dynamics and suppression of viral replication in vitro by Gag-specific CD4+ T-cell clones with antiviral effector properties similar to those characteristic of antiviral CD8+ T-cell clones, consistent with a cytotoxic CD4+ T cell phenotype. Because these antiviral CD4+ T cell clones suppress viral replication among target cells yet are susceptible to infection themselves, the RCIS examined the competing dynamics between virus suppression in CD4+ T-cell targets and the levels of SIV infection in the effectors themselves. The results indicate that, unlike CD8+ T cells, antiviral CD4 T cells need to be able to adequately suppress infection among themselves so that they can be effective in reducing SIV replication in their corresponding targets. To enable future studies of this poorly understood portion of the overall antiviral cellular immune response, the RCIS also generated an MHC class II-restricted TCR transfer vector by cloning the TCR from a CD4+ T cell clone with potent in vitro antiviral activity. The cloned TCR represents a renewable resource allowing generation by transfection of essentially unlimited additional populations of cells expressing this TCR for future studies.

Retroviral Pathogenesis Section

The Retroviral Pathogenesis Section (RPS) studies interactions of primate lentiviruses with their hosts to better understand the mechanisms by which these viruses cause disease, in order to more effectively prevent and treat such infections and their consequences.

SIGNIFICANT ACHIEVEMENTS

In Vitro Studies: In collaborative studies with Mary Carrington of the Basic Research Program, LBRI/FNLCR, the RPS demonstrated that, in contrast to longstanding but under-substantiated conventional wisdom in the field, HIV infection does indeed result in downregulation of HLA-C, most frequently via a viral Vpu protein–mediated mechanism, a finding with potential to help explain differential HIV-1 pathogenesis in different infected individuals (Apps et al., *Cell Host Microbe*, 2016,19:686-95).

Improved SHIV/NHP models: Chimeric viruses containing HIV-1 Env sequences in a SIV backbone, designated SHIVs, are a mainstay of research on Env-targeted prophylactic and therapeutic interventions, including vaccines, antibodies, and other approaches, but existing SHIVs in the field are not representative of current, clinically transmitted viruses that are the intended target of these interventions. In collaboration with Drs. Paul Bieniasz and Theodora Hatziioannou (Rockefeller University/Aaron Diamond AIDS Research Center) and with Dr. George Shaw (University of Pennsylvania), the RPS developed strategies for generating and evaluating novel SHIVs that embody a range of clinically relevant features, including R5 tropism, the use of transmitted Env sequences, and tier 2 neutralization phenotypes. Encouraging results have been obtained with SHIVs in collaborative studies with Dr. Shaw (Li et al., *Proc Natl Acad Sci USA, 2016*, 113: E3413), while in vivo evaluations of several additional SHIVs, including viruses incorporating transmitted Clade C Env sequences, are in progress in collaboration with Dr. Keele of the RES/ACVP and investigators from the NIAID Vaccine Research Center.

Viral "reservoirs" and "HIV Cure" research in NHP models: Characterizing and devising definitive interventions to target the residual virus that persists in the face of cART is one of the frontier areas of AIDS research. The RPS has pioneered the development of NHP models for studies relevant to this challenge. The group continues to lead the development and application of novel, effective, and sustainable cART regimens for use in NHP, including effective regimens that have been continuously dosed for nearly three years. (Del Prete et al., *AIDS Res Hum Retrovir*, 2016, 32:163). These regimens are being used to evaluate various different approaches to interruption of AIDS virus latency in NHP on suppressive cART, including with histone deacetylase inhibitors (Del Prete et al., *Antimicrob Agents Chemother*, 2015 60:1560), and toll-like receptor ligands (Del Prete et al., in preparation). Therapeutic vaccination approaches are also under evaluation, along with collaborative studies with Dr. Ott (RCIS/ACVP) involving adoptive transfer of CD8+ T cells engineered to express SIV-specific T cell receptors and homing receptors, including CXCR5 to facilitate more effective trafficking to viral reservoirs such as infected T follicular helper cells in B-cell follicles of secondary lymphoid tissues.

Viral Oncology Section

The overall aim of the Viral Oncology Section (VOS) is to study the role of viruses in cancer. Studies are focused in three major areas: Kaposi's sarcoma-associated herpesvirus (KSHV) epidemiology and transmission, KSHV immunity and pathogenesis, and viral and host genetics in KSHV infection and disease.

SIGNIFICANT ACHIEVEMENTS

Project 1: KSHV Epidemiology and Transmission: We have continued our KSHV research program in Uganda in collaboration with Uganda Virus Research Institute (UVRI) in Entebbe, Uganda. In January 2016, Drs. Whitby and Labo visited the UVRI and the Uganda General Population Cohort (UGPC) field station in Kyamulibwa. In addition to gaining insights into the settings of the ongoing studies, considerable progress was made on analyses of current data, and a number of manuscripts have been submitted or are in preparation as a result. These include a manuscript on risk factors for cancer in rural Uganda; KSHV antibody levels in the UGPC during the HIV epidemic; and a longitudinal analysis of KSHV antibodies in children in the Entebbe Mother and Baby study (EMaBS). An analysis of KSHV viral load in saliva of KSHV-infected mothers and children in Uganda was extended to include EBV viral load, enabling a comparative analysis of factors associated with shedding of these related oncogenic viruses in a setting where both childhood KS and endemic BL are relatively common. An oral presentation was made of the data at the 15th International AIDS Malignancy Workshop, Bethesda, in November 2015 and at the 4th Emerging Issues in Oncogenic Virus Research Workshop, Manduria, Italy, June 2016. A manuscript is in preparation.

Further studies related to the Cameroon KS case control studies are ongoing, including analysis of risk factors associated with KSHV infection and detectable viral load in saliva and blood, including total IgE as a surrogate for total parasite burden. We continue to develop our collaboration with Dr. Rosemary Rochford to study cohorts of mothers and children in Kenya. Collaborations have been developed with additional investigators in sub-Saharan Africa. We have successfully transferred our KSHV ELISA assays to two groups in South Africa, Dr. Shafer in Cape Town and Dr. Bessong in Limpopo, thus enabling KSHV research by local investigators in more regions where KSHV is endemic. Analyses are also ongoing of KSHV and KS in the MACS cohort.

Project 2: KSHV Immunity and Pathogenesis: The VOS has extended its ambitious project to systematically investigate the immunogenicity of the KSHV proteome. The initial screening panel was limited in size and diversity. Using the flexibility of luminex bead–based technology, VOS has developed an assay using 68 antigens to study KSHV antibody responses in depth. VOS has now extended studies of antibody reactivity to

KSHV to populations in sub-Saharan Africa. VOS has also studied longitudinal trends in KSHV antibody responses, including in sero-converting children in three cohorts in Uganda, Kenya, and Zambia. The VOS has developed a collaboration with Dr. Ruth Pfieffer of the DCEG Biostatistics Branch to explore novel statistical approaches to analyses of these complex data. The VOS has also developed a collaboration with the UK biobank project and the University of Heidelberg to validate KSHV assays for use in the UK biobank project. The VOS has continued to provide reagents generated during the KSHV proteome project to researchers in the KSHV field, and this year, two manuscripts were published based on such interactions with Dr. Fanxiu Zhu of Florida State University, demonstrating the utility of these reagents to the KSHV field.

Major progress has been made on development of an ELISPOT assay to detect cellular immune responses to KSHV using overlapping pooled peptides for the entire KSHV genome. KSHV responses in healthy KSHV seropositive donors and patients with KSHV-related diseases have been mapped. A project to make and characterize T-cell clones from subjects with positive responses is ongoing in collaboration with Dave Ott. Two manuscripts are in preparation and a poster was presented at the AIDS Malignancy Workshop.

The VOS had previously reported genetic variation in the KSHV microRNA encoding region in cell lines and clinical samples. Studies are ongoing to determine the microRNA profiles of KS biopsies from South Africa and the U.S. from subjects whose microRNA region sequence we also determined. These studies, using a combination of array and real time PCR approaches, will enable us to demonstrate the effect of sequence variation on microRNA expression in clinical KS. The VOS has collaborated with Dr. Stuart Le Grice to incorporate 2-D and 3-D RNA structural techniques into this project. A poster describing this work was presented at the KSHV meeting and a manuscript is in preparation. In addition, a manuscript describing a related study of host microRNAs induced by KSHV infection was published in collaboration with Dr. Rolf Renne, University of Florida.

The VOS maintains CLIA certification and has continued to collaborate with the HIV and AIDS Malignancy Branch on clinical projects to understand basic KSHV pathology and also to evaluate novel therapeutic approaches for KSHV related disease. The number of samples processed according to CLIA continues to increase.

Project 3: Viral and Host Genetics in KSHV Infection and Disease: Using KS cases and KSHV positive controls from the Cameroon case control study, a project is ongoing to determine host and viral genetic factors that play a role in KSHV transmission and KS pathogenesis. A panel of host immune and viral receptor genes has been selected for targeted exome sequencing and a Sureselect probe set designed. Sequencing has been completed for approximately 200 KS cases and HLA matched controls. Analyses are ongoing and have identified loci possibly associated with KS risk amongst HIV-infected men in this population. Within the same study, we are also investigating HLA and KIR sequence diversity in collaboration with Mary Carrington, in order to determine if variation in these central adaptive/innate immune molecules influence KSHV transmission and/or disease pathogenesis. HLA typing has been completed for approximately 500 KS cases and controls, and posters have been presented by Dr. Elena Cornejo Castro at the Spring Research Festival and the 19th KSHV workshop. A manuscript has been published in collaboration with Dr. Carrington on HLA and risk of HIV-negative KS risk in a prior study. We have also collaborated with Dr. Ines Barroso at the Sanger Institute UK and Dr. Robert Newton UVRI, Uganda, to examine genetic variations associated with high antibody levels to KSHV and EBV in the UGPC via GWAS. A manuscript on genetic markers of high EBV antibody levels is currently under consideration.

We have used next-generation sequencing to determine entire KSHV genome sequences from clinical and epidemiological samples. Shotgun Illumina sequencing has been used to derive whole KSHV genomes from 15 Pleural effusions samples with extremely high KSHV viral loads in collaboration with Drs. Brandon Keele and Robert Yarchoan. This work was presented at the AIDS Malignancy Workshop, and a manuscript is in preparation. Using a targeted resequencing approach, in collaboration with the Sanger Institute, UK, we have developed a SureSelect probe set to capture KSHV and EBV genomic sequences from samples from the EMaBs cohort, the UGPC, and the Cameroon KS Case Control Study. Initial protocol development has been completed, and sequencing is ongoing.

In addition to investigator-initiated research conducted within the ACVP, in keeping with the mission of FNLCR, the eight research support cores of the ACVP also provide useful and unique products and services to the broader research community, including both intramural and extramural investigators.

Retroviral Immunopathology Section

The Retroviral Immunopathology Section (RIPS) conducts a broad range of basic and applied research, focusing on in vivo studies in NHPs, with the common theme of studying viral–host interactions in tissue compartments to better understand the mechanisms and processes by which lentiviruses drive immunopathology and cause disease. The mission of the RIPS is to study the host–viral interactions in vivo to better elucidate: (i) the sources and mechanisms driving systemic inflammation and immune activation; and (ii) the processes underlying lentiviral mucosal transmission, viral dissemination, reservoir establishment, and viral persistence. The principal objective is to translate our improved understanding of disease processes in vivo to design and/or strategically and effectively target key pathways

driving immunopathology and/or viral persistence through adjunctive therapeutic interventions and prevention modalities, goals that are well aligned with the mission of both the ACVP and the FNLCR.

SIGNIFICANT ACHIEVEMENTS

During FY2016, the RIPS made significant progress in both our primary areas of research interest. We are currently finalizing a manuscript on studies initiated by Drs. Estes and Keele aimed at determining the barriers and early events leading to vaginal and rectal transmission, in order to understand the process of viral reservoir establishment (collaboration with Drs. Keele [RES] and Lifson [RPS]). Dr. Estes additionally wrote two review articles highlighting his expertise in imaging lymphoid tissues to understand HIV/SIV pathogenesis and persistence (*Curr HIV/AIDS Rep,* 2016 Feb;13(1):38-43; *Curr Opin Virol*, 2016. In Press).

The RIPS also recently published a study in *JCI Insight* that showed the magnitude of GI tract damage, immune activation, and inflammation was significantly increased with significantly depleted $CD4^+$ T cells in the lamina propria (LP), even in acutely HIV-infected individuals identified at the earliest time clinically feasible, compared to uninfected control individuals. The results demonstrate the rapid and profound impact of HIV infection within the GI tract early after acquisition. While most study participants treated during early acute infection resolved GI tract inflammation and immune activation back to baseline levels after only 24 weeks of cART, most did not restore $CD4^+$ T cells in the LP to normal levels, even after 96 weeks of cART (*JCI Insight*, 2016;1(10):e87065).

The RIPS also developed a specific and sensitive next-generation in situ hybridization approach to detect vRNA, including vRNA+ cells and viral particles ("RNAscope"), vDNA+ cells ("DNAscope") and combined vRNA and vDNA with immunohistochemistry to detect and phenotype active and latently infected cells in the same tissue section. In a manuscript published in *Pathogens and Immunity*, the RIPS, working with the TAC, used these new methods to track and discriminate viral reservoirs within tissue compartments. We demonstrated that RNAscope is highly sensitive with greater speed of analysis compared to traditional in situ hybridization approaches. In addition, we showed that the highly sensitive and specific DNAscope approach detected SIV/HIV vDNA+ cells, including duplexed detection of vDNA and vRNA or immunophenotypic markers in the same section. Analysis of lymphoid tissues samples from NHPs prior to and during cART using these new in situ approaches demonstrated that B-cell follicles are an important anatomical compartment for both latent and active viral persistence during treatment. The development of these new tools by the RIPS should allow new insights into viral reservoir biology and evaluation of cure strategies (*Pathog Immun*, 2016, **1**: 68-106).

Finally, in a continuing collaboration with the NCI Molecular Imaging Program, we completed an 88-week animal study that longitudinally quantified the impact of progressive SIV-induced lymph node fibrotic damage on antigen uptake using a non-invasive MRI approach. This MRI approach will be correlated to detailed histological assessments of lymphoid tissue fibrosis in tissues collected during the study as well following the completion of the study.

Retroviral Evolution Section

The Retroviral Evolution Section (RES) is dedicated to addressing essential research questions encompassing (i) viral transmission (ii) viral adaptation to host immunity, and (iii) viral reservoir establishment and maintenance. We have made significant advances in 2016 with eight published manuscripts and several additional studies completed with manuscripts in preparation.

SIGNIFICANT ACHIEVEMENTS

The first project for the RES is built upon the hypothesis that the greatest opportunity for inhibiting viral growth occurs during the transmission process, when the viral population is significantly reduced to one or a few variants. This genetic bottleneck highlights a significant weakness of the viral life cycle. However, the mechanisms and details surrounding the transmission process and early viral replication dynamics are poorly understood. The RES completed two serial-timed necropsy studies in rhesus macaques aimed at identifying the genetic bottleneck following vaginal and rectal exposure to SIV. We have identified the route of systemic infection, enumerated the number of variants within the sites of viral exposure, and determined a variable timing to dissemination. We have adapted MiSeq sequencing and a molecularly tagged virus, providing an unprecedented view into the transmission processes. Both the vaginal and rectal studies are completed and are currently being submitted for publication. Additionally, we collaborated with Dr. Estes (ACVP, RIPS) to show experimentally induced colitis reproduces important features of pathogenesis (Hao et al., *Nat Comm*, 2015) that models CD4+ T cell loss and microbial translocation initiated during primary infection.

The RES has several research projects assessing viral adaptation to host immunity and model development. Currently available SIV infectious molecular clones (IMCs) and isolates used in NHP models of AIDS were originally derived from infected macaques during chronic infection or end-stage disease and may not authentically recapitulate features of transmitted/founder (T/F) genomes that are of particular interest in transmission, pathogenesis, prevention, and treatment studies. We therefore generated and characterized T/F IMCs from genetically and biologically heterogeneous challenge stocks of SIVmac251 and SIVsmE660. Single-genome amplification (SGA) was used to identify full-length T/F genomes present in plasma during acute infection resulting from atraumatic rectal inoculation of Indian rhesus macaques with low doses of SIVmac251 or

SIVsmE660. All eight T/F clones yielded viruses that were infectious and replication competent in vitro, with replication kinetics similar to the widely-used chronic-infection derived IMCs SIVmac239 and SIVsmE543. Phenotypically, the new T/F virus strains exhibited a range of neutralization sensitivity profiles. Four T/F virus strains were inoculated into rhesus macaques, and each exhibited typical SIV replication kinetics. All T/F viruses were pathogenic in rhesus macaques, resulting in progressive CD4+ T cell loss. Animals developed pathological immune activation, lymphoid tissue damage including fibrosis, and clinically significant immuno-deficiency leading to AIDS-defining clinical endpoints (Lopker et al., *J Virol*, 2016). We expanded this work in collaboration with Dr. Shaw (University of Pennsylvania) utilizing one of our new molecular clones as a backbone for a novel approach in developing HIV Envelope SHIV clones. In this study, a mutation at position 375 of Envelope mediates increased rhesus CD4 binding. This mutation in Envelope allows for increased viral replication in rhesus macaques within the context of a transmitted/founder backbone SIVmac766 (Li et al., *Proc Natl Acad Sci*, 2016). In addition, we collaborated with Dr. Katie Bar (University of Pennsylvania) to assess viral evolution and neutralization profiles of SIVsmE660 clones following vaccination (Lee et al., *J Virol*, 2015). Additionally, with Dr. Li (University of Nebraska), we assessed the viral adaptation of SIVcpz following infection of humanized mice (Yuan et al., *J Virol*, 2016).

Another research project within RES seeks to answer key scientific questions of reservoir establishment and maintenance utilizing cART regimens developed by Dr. Lifson (Del Prete et al., *AIDS Res Hum Retrovir*, 2016). We have designed a novel sequence tagged virus system to track individual viral reservoirs. This virus model uses a virally encoded barcode to genetically distinguish individual viral lineages. We developed, characterized, and validated the barcoded virus approach in both untreated and cART-treated NHPs. We determined that our virus stock contains 10,000 unique genomes (SIVmac239M) that were distinguishable by sequence analysis following next-generation sequencing implemented on MiSeq instrument. We have completed our primary NHP study to examine the effects of the timing of cART initiation on reservoir size. Animals were infected and antiviral therapy initiated at day four, and treatment continued for up to 480 days before interruption to assess viral rebound kinetics and to enumerate the number of rebounding variants. Between three and 63 variants were found following viral rebound. Individual barcodes found at rebound were distributed throughout the range of the different variants in the viral stock and found in animals prior to therapy. The estimated reactivation rates ranged from 0.38–0.7 events per day. Peak viral load at time of treatment initiation was a significant predictor of frequency of reactivation. Once cART was initiated and viral replication limited, additional time on therapy did not reduce the number of rebounding genomes or decrease reactivation rates, suggesting a stable reservoir in NHPs can be achieved, which recapitulates HIV infection in humans. This study is in preparation for publication, and this technology has spurred additional projects and collaborations with intramural and extramural investigators.

Support Provided by the Laboratory Animal Sciences Program

Animal Research Facilities Operations

National Cancer Institute (NCI) at Frederick and NCI at the Bethesda campus are among 750 organizations, institutions, and companies in 29 countries that are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

The Laboratory Animal Sciences Program (LASP) manages 27 animal research facilities, 22 of which are located in Frederick and five in Bethesda. Collectively, the facilities on both campuses support 223 investigators (90 in Frederick and 133 in Bethesda) and 554 active Animal Study Proposals (ASPs) (189 in Frederick and 365 in Bethesda), and are currently maintaining 129,191 animals (83,264 in Frederick and 45,927 in Bethesda) occupying 41,078 cages. The animal support areas of LASP include: NCI animal facilities, the Animal Health Diagnostic Laboratory (AHDL), Receiving and Quarantine (R&Q), and Laboratory Animal Medicine (LAM). LASP serves primarily as a resource for animal-based research for the NCI scientific community. Additionally, LASP provides significant levels of support to other institutes and entities, including the National Institute of Allergy and Infectious Diseases (NIAID), Leidos Biomedical Research, Inc. programs, and other agencies approved by NCI. In addition to facility operations, LASP provides a wide array of support services in a number of areas, including colony management, technical support on an as-needed basis, requested dedicated technical support; and performance of, as well as training in, advanced surgical procedures. The current animal population is distributed as follows:

Species	Animals
Mice	124,827
Rats	398
Frogs	158
Nonhuman Primates	184
Fish	3624
Total	**129,191**

Significant Achievements – Bethesda

LASP continued to offer on a monthly basis its well-received, in-house, hands-on training program in aseptic surgery techniques in which 83 NCI research staff have participated to date.

LASP successfully initiated an Assistant Laboratory Animal Technician (ALAT) training course that resulted in eight LASP staff members who attended the course achieving eligibility to take the certification examination. During this period, three LASP staff successfully passed the AALAS certification exams: two at the ALAT and one at the Laboratory Animal Technician (LAT) level. Three more are scheduled to sit for the examination next month.

LASP staff participated in the NCI-wide Mouse 101 course for NCI investigators, presenting over 4 hours of instruction on animal care and use topics, which was simultaneously webcast to investigators in both Bethesda and Frederick. In addition to the lecture component, LASP conducted six hands-on training classes for 30 NCI investigators on animal handling and use techniques using live animals.

LASP enhanced the daily animal health-status communications with investigators through the use of iPads in LASP-managed rodent facilities. This has resulted in better, more complete health reports to investigators, faster communication between LASP staff and investigators, and saved several man hours each day. This new initiative has been warmly received by research staff and received accolades on recent monitors report.

LASP management initiated biweekly meetings with the NIH Office of Research Facilities to enhance communication which has resulted in expeditious repairs for the many animal facility issues during the past year.

LASP initiated quarterly facility users' meetings for each of the LASP managed animal facilities, which has facilitated better communication and has been well received by NCI investigators.

LASP initiated the ACUC review and approval of all current Standard Operating Procedures which has streamlined animal protocol preparation.

LASP successfully managed the adverse impact on facility-wide animal husbandry operations during several challenging periods: 1) the two-month repair of the walk-in autoclave in the Clinical Research Center (CRC) vivarium; and 2) over four months of both high temperature and humidity in both the 14DS nonhuman primate facility and Building 41 rodent facility. Through exceptional coordination and management, all animal cages were transported to either other LASP-managed animal facilities for sanitation and returned to the CRC (#1) or relocated within the facility with support fans and the like, no animals died.

The ACUC administrators, Rebekah Smith and Olga Kuznetsova, greatly contributed to the revision and upgrading of the iRIS animal protocol program through weekly meetings with both the ACUC chairs and the Center for Cancer Research (CCR) IT director as well as actively testing each revision prior to releasing it for principal investigator (PI) use. In addition, and on her own initiative, Smith started monthly training sessions for PIs to assist them in navigating this often very difficult animal protocol system.

LASP successfully managed the adverse impact on facility-wide animal husbandry operations during the two-month repair of the walk-in autoclave in the CRC vivarium. Through exceptional coordination, all animal cages were transported to other LASP-managed facilities for sanitation and returned to the CRC such that no animal health status was compromised.

Fifteen LASP animal husbandry staff successfully completed the two- semester twice weekly Montgomery College Course (with 100% attendance): ESOL Civic English Speaking Second Language course which has greatly enhanced communication between LASP staff and investigators.

LASP veterinarians participated on Animal Research Advisory Committees developing NIH-wide guidelines for both rodent surgery and personnel protective equipment.

Dr. Robert Hoyt was selected as one of two NIH Institute Animal Program Directors (APD) to serve on the NIH–wide Animal Facility Project Advisory Board (AFPAB) Committee which determines funds for facility improvements for NIH–wide institutions.

Dr. Hoyt was reappointed as an adjunct professor at the Virginia-Maryland College of Veterinary Medicine.

Significant Achievements – Frederick

Jennifer Wise, BS, started as the manager for Building 567 in January 2016 to replace Rhonda Anderson after her retirement.

Facility upgrades initiated and successfully conducted by LASP in Frederick include the following:

- Animal Production Area (APA) paving phase II and III completed.
- Building 571 floor repairs completed.
- Building 539-2 autoclave replacement completed.
- Building 571 exterior replacement completed. Worked extensively with FME for noise/vibration monitoring and control. This was a good learning tool for future construction projects.
- Building 1021 and Building 1046 roofing replacement completed.
- Building 539/Room 265 biological safety cabinet exchange completed.
- Building 539 install exterior insulation finish system (EIFS) completed.
- Building 1046 autoclave replacement completed.
- Building 539/Room 261 Class II/B2 BSC installation completed.
- Building 539 sewer/paving completed.
- Building 550 emergency backup power generator installation completed.
- Building 550 reverse osmosis (RO) water upgrades completed.
- Building 539/Rooms 193/194 renovation completed. Old blender hoods and chemical fume hoods were removed and replaced with class IIB2 hoods.

- Building 1075 chemical hood swap with BSC completed.
- Building 567 animal room pneumatic controls replacement completed.
- Buildings 1029, 1036, and 1037 high-efficiency particulate air (HEPA) filter exhaust installation completed.
- Building 376 MRI suite to accommodate new Oxford hyperpolarizer renovation completed. Installation expected in August, 2016.
- Building 539-1CC installation of biological research X-ray irradiator completed.
- Campus-wide ground fault circuit interrupter (GFCI) installation complete.
- Energy conservation
  - Upgrading to LED light bulbs completed in all animal facilities.
- Building 567 heat exchanger replacement completed.
- Building 571 chiller replacement completed.
- Working with Facilities Maintenance and Engineering (FME) and Environment, Health and Safety (EHS) to replace the existing portable generators with a single permanent generator to provide emergency power in case of outage at APA animal facilities.
- Building 567 cage wash exhaust and supply air handling unit replacement is in progress.
- Buildings 1035 and 1025 renovations (installation of new autoclaves) anticipated to be completed by mid-August, 2016. Building 1025 will be used to provide additional animal housing space for Division of Cancer Treatment and Diagnosis (DCTD). Building 1035 will be used to house cryo animals from Building 550 to allow Building 550 to be dedicated to the gnotobiotic program.
- Buildings 571 and 539-1CC tunnel washer replacement project in progress and anticipated to be completed by October, 2016.
- Building 539-1CB rebuild BetaStar autoclave with new piping and controls in progress.
- Building 550 installation of air flow alarms for germ free isolators is in progress.
- Buildings 539-1CB and 1021 addition of bedding dispensers are in progress—anticipated completion date September, 2016.
- Building 539 first floor renovation project to convert non-animal space to animal space is in the design phase. The anticipated start date of the conversion is July, 2017.
- Building 325 designing and renovation in progress. Building 325 will be used as the transition space for Building 539 during renovation period (3–5 years) starting January, 2017.

Animal Health Technical Support Group

The LASP Animal Research Technical Support Group provides customized technical support for basic and translational animal-based research to NCI's scientific community. Services range from expert colony management to the performance and development of technical procedures aimed at disease induction, characterization, and treatment of animal models. The team facilitates research projects by working closely with the principal investigators throughout all stages of their projects by providing assistance with animal study proposals and IBC registrations, developing highly-detailed study setup sheets, coordinating and scheduling experimental studies; developing, implementing, and carrying out all aspects of studies, including advanced surgical procedures; and providing regular progress reports and delivering detailed analysis results. All tasks within the workflow are conducted in accordance to all pertinent regulations (ACUC, IBC, and NRC) and coordinated with other LASP core facilities to ensure timely transfer of animals and study information between groups.

Significant Achievements

The Animal Research Technical Support Group has continued to manage complex breeding colonies of genetically modified mouse models and rats, including the generation of timed matings as well as experimental cohorts and monitoring of phenotypes associated with diseases in support of 16 Center for Cancer Research (CCR) investigators and National Center for Advancing Translational Sciences (NCATS). The team has prepared 194 chemicals for dosing and treatment of animals as well as medicated water or diets for 15 CCR investigators and NCATS.

The Animal Research Technical Support Group conducted 104 experimental studies comprising of bone marrow transplants, tumor implantations, pharmacokinetics (PK)/pharmacodynamics (PD)/toxicity and efficacy studies on mice and rats in support of the following programs/divisions:

CCR/NCI (88 studies)

- Basic Research Laboratory (6 studies)
- Chemical Biology Laboratory (2 studies)
- Cancer and Developmental Biology Laboratory (10 studies)
- Cancer and Inflammation Program (1 study)
- Laboratory of Cancer Biology and Genetics (8 studies)
- Laboratory of Cellular and Molecular Biology (8 studies)
- Laboratory of Molecular Biology (1 study)
- Laboratory of Protein Dynamics and Signaling (10 studies)
- Mouse Cancer Genetics Program (15 studies)
- Urologic Oncology Branch (7 studies)
- Women's Malignancies Branch (20 studies)

CSSI/NCI

- Nanotechnology Characterization Laboratory (9 studies)

NCI (Yellow Tasks [YT])

- Division of Cancer Prevention (1 study)
- Technology Transfer Center Invention Development Fund (1 study)

NCATS/NIH (YT)

- Leopard Syndrome (5 studies; 2 animal models)

In addition, the group has provided technical assistance for tumor implantations, resections, and treatments on projects run by the Small Animal Imaging Program (SAIP) in support of the Molecular Imaging Program and the Cancer Imaging Program as well as experimental studies in the Gnotobiotics Core Facility.

Gnotobiotics Core Facility

The Gnotobiotics Core Facility (GCF) has been reorganized as a separate core under Animal Research Technical Support and supports research efforts that are focused on the role of microbiota in inflammation, pathogenesis, and anti-tumor response. It is presently outfitted with 6 breeder isolator units and 12 experimental isolator units. Services offered include the rederivation, breeding, and conducting of experimental studies on germ-free and gnotobiotic mice. Within the next fiscal year, the facility will be expanded to increase the experimental and breeding capacity.

SIGNIFICANT ACHIEVEMENTS – FREDERICK

GCF successfully performed the rederivation of two mouse strains into the germ-free state and is offering this service to CCR investigators. Colonies of five germ-free mouse strains were established, and animals are being bred for use in experiments by CCR investigators. LASP staff conducted 13 experimental studies, including tumor cell injections and cancer treatments for investigators from the Cancer Inflammation Program and provided animals for experimental purposes to investigators from the Dermatology Branch.

Animal Health Diagnostic Laboratory

The AHDL is responsible for monitoring the health of all rodents at the NCI at Frederick and NCI's Bethesda campus animal facilities, and it provides diagnostic resources to several other NIH facilities. The major focus of the diagnostic services includes microbiology, parasitology, serology, and heath-monitoring necropsies. The AHDL has been a consistent resource to the scientific community for more than 40 years.

SIGNIFICANT ACHIEVEMENTS – FREDERICK

- During this reporting period, AHDL performed 2,382 necropsies, 60,464 serological tests, 9,560 bacteriological tests, 14,504 ectoparasite tests, and 21,398 endoparasite tests as part of its routine health monitoring function.
- AHDL continues to provide environmental monitoring of the isolators and supplies for the Gnotobiotic Core Facility. This additional effort often requires the AHDL to turn results around without delay to ensure the timely processing of required supplies to support germ free isolator operations. The AHDL detected microbes on the supposed clean mice procured from vendor that saved precious time and cost for investigator should these animals be used in germ-free experimentation.
- The AHDL detected fur mite ova in a group of imported quarantine mice. The room was quarantined, the positive mice transferred to a conventional health status room, and other groups of mice in the room were treated for fur mites. The AHDL screened mice that were transferred from this room to the animal facilities to ensure no fur mites were found.
- The AHDL is also providing monthly diagnostic support for routine health monitoring associated with the isolator colonies maintained for NCI's Developmental Therapeutics Program (DTP).

Receiving and Quarantine Program

The Receiving and Quarantine (R&Q) Program is a vital, gate-keeping facility that is charged with safeguarding and protecting the health status of the Frederick National Laboratory for Cancer Research (FNLCR) and NCI's Bethesda campus rodent colonies. R&Q is responsible for the quarantine of imported animals that may harbor unwanted viral and microbial agents, and for coordination of animal shipments.

SIGNIFICANT ACHIEVEMENTS

Approximately 96 animal importations were processed, of which 2 were from modified-approved sources, and 94 were from non-approved sources.

Byron Bowie III began employment as the dedicated laboratory animal technician for R&Q. LASP R&Q staff assumed responsibility for rooms in Building 429 housing animals destined for rederivation.

The importation process was streamlined, removing the necessity for approval of bypass imports, and an additional new approved vendor, Envigo, was added. A list of approved vendors is updated and released monthly to all LASP administrators responsible for animal ordering.

Laboratory Animal Medicine Program

The Laboratory Animal Medicine (LAM) program is organized to: (1) provide research support to NCI investigators, (2) ensure the welfare of NCI animals, and (3) ensure compliance with all local, state, and federal regulations that govern the ethical use of animals in biomedical research, with the continuing objective of maintaining full compliance with AAALAC International.

LAM provides a wide array of support services to NCI investigators, including assisting with the development, submission, and modification of Animal Study Proposals (ASPs); consulting and training on surgical procedures, anesthesia, analgesia, endpoints, and various technical procedures; and developing, performing, and refining research, surgical, and diagnostic techniques for animal models.

LAM also provides a comprehensive array of support services to NCI animal programs, including provision of high-quality medical, surgical, and behavioral support; oversight of biosecurity, animal procurement, and sentinel programs; establishment of policies, practices, and operating procedures to ensure regulatory compliance; and acquisition of supplies requiring veterinary prescriptions.

LAM provides support and guidance to FNLCR ACUC and IBC, oversees FNLCR animal facilities, and organizes the American Association for Laboratory Animal Science (AALAS) training courses for technicians.

SIGNIFICANT ACHIEVEMENTS

Dr. Jatinder Gulani, DVM, DACLAM, began employment with LASP as senior animal program veterinarian on October 26, 2015.

LAM veterinary staff and ACUC coordinator reviewed and processed 52 animal study protocols, 410 modifications, and 4 policies for FNLCR animal care and use committee.

LAM veterinary staff and Quality Assurance coordinator revised/created 15 standard operating procedures (SOPs) and conducted 15 SOP training sessions for LASP staff.

An updated Animal Health Overview was developed and is currently being implemented, in-person, to all LASP staff.

The Animal Health Evaluation, Reporting, and Treatment SOPs was updated with help from all departments leading to a new and streamlined Health Check card, reporting, and treatment system.

LAM personnel performed approximately 20 Post-Approval Monitoring sessions in addition to numerous training sessions on techniques such as basic handling and restraint, euthanasia, injections, intratracheal instillation, etc.

LAM initiated quarterly training sessions for all the husbandry and technical staff in January, 2016. These sessions were used for providing training on changes in SOPs and policies.

LAM personnel approved two new trainers, Amy James and Christina Robinson, for multiple surgical and nonsurgical techniques.

LAM personnel, Jatinder Gulani, Gillian Braden, Blayre Montague, Christina Robinson, and Julie Bosworth, completed the aseptic technique in rodent surgery course taught by Dr. Robert Hoyt.

Bosworth began teaching weekly AALAS certification classes at the ALAT, LAT, and LATG levels.

AALAS certification training and preparation facilitated by LAM resulted in one LAT and two ALAT certifications in Frederick; and one LAT certification in Bethesda.

Scientific Support Programs

Mouse Model Core Technology Laboratory

The Mouse Model Core Technology Laboratory is a comprehensive program where the mouse models are generated and the characterized models are ultimately archived. The laboratory comprises the following functional components: transgenic mouse models (TMM), cryopreservation and assisted reproduction, cell culture, and finally the rederivation service, which is housed within the Receiving and Quarantine facility.

Transgenic Mouse Model Laboratory

The primary role of the Transgenic Mouse Model Laboratory (TMML)is the production of genetically engineered mice by gene transfer into developing embryos (pronuclear microinjection), which has been instrumental in the study of in vivo gene function through the use of genetically engineered mice to model human diseases. Transgenic mouse models have specifically been essential for investigating gene functions and their role in cancer biology. The TMML offers a complete array of services aimed at successfully generating transgenic and gene-targeted mice. For production of transgenic mice, the services include consultation with the investigator regarding transgenic design; purification of the DNA fragment to be microinjected; pronuclear microinjection of DNA into fertilized mouse oocytes; and genotypic evaluation of the resulting animals by Southern blot analysis. The gene-targeting component of TMM is a comprehensive service that involves advising the requesting investigator on the design of the targeting vector, targeting the mouse embryonic stem cells (mESC) by electroporation, selecting the correctly targeted mESC clones by Southern blot hybridization, and generating chimeras by microinjection of karyotypically normal targeted embryonic stem (ES) cells, which is followed by confirmation of germline transmission of the targeted mutation. The TMM laboratory also performs microinjection of mutant ES cells obtained from repositories such as the European Conditional Mouse Mutagenesis Program (EUCOMM) and the microRNA (miRNA) Resource available through the NCI Mouse Repository. Chimeras generated will be transferred to the requesting investigator for further characterization.

SIGNIFICANT ACHIEVEMENTS

The TMM laboratory is anticipated to successfully complete 49 construct-based transgenic, knock-out, conditional knock-out, and knock-in mouse models, in support of 18 principal investigators; this is a decrease from the 62 projects completed in FY2015. Included are 13 projects utilizing the CRISPR/Cas9 technology,

implemented as a routine service by the Mouse Model Core Technology Laboratory for NIH's scientific community, in support of Drs. R. Hodes, NCI (6 projects), A. Bhandoola, NCI (5 projects), and F. Gonzalez, NCI (2 projects), and 1 project utilizing TALENs to generate a knock-in mouse model in support or Dr. Ellis, NCI. In addition to NCI, TMM provides services for other institutes within NIH. The table below lists the investigators/ programs that have requested support services from TMM for production of mouse models for their studies.

Principal Investigators Who Used Transgenic Mouse Models September 26, 2015 through September 25, 2016

Principal Investigator	Laboratory	Number of Constructs
Dr. P. Aplan	Cancer Genetics Branch, NCI	1
Dr. R. Casellas	Molecular Immunology and Inflammation Branch, NIAMS	1
Dr. F. Gonzalez	Laboratory of Metabolism, CCR, NCI	5
Dr. R. Hodes	Experimental Immunology Branch, CCR, NCI	6
Dr. Y. Belkaid	Mucosal Immunology Branch, NIAID	1
Dr. H. Park	Experimental Immunology Branch, CCR, NCI	3
Dr. R. Sen	Laboratory of Molecular Biology and Immunology, NIA	2
Dr. A. Singer	Experimental Immunology Branch, CCR, NCI	7
Dr. I. Pastan	Laboratory of Molecular Biology, CCR, NCI	1
Dr. A. Bhandoola	Laboratory of Genome Integrity, CCR, NCI	5
Dr. K. Ellis	Section of Developmental Neuroscience, NIDCD	1
Dr. N. Restifo	Surgery Branch, CCR, NCI	2
Dr. B. McCright	Center for Devices and Radiological Health, FDA	1
Dr. L. Zhang	Section of Synaptic Pharmacology, NIAAA	1
Dr. L. Dong	Genetic Engineering Core, NEI	2
Dr. S. Gara	Endocrine Oncology Branch, NCI	2
Dr. T. Badea	Retinal Circuit Development & Genetics unit, NEI	4
Dr. V. Lazarevic	Experimental Immunology Branch, CCR, NCI	1
Dr. J. Farber	Laboratory of Molecular Immunology, NIAID	1
Dr. C. Harris	Laboratory of Human Carcinogenesis, CCR, NCI	2
Total		**49**

Among the projects listed above, 16 requests were for microinjection of genetically engineered ES cells on various parental backgrounds (KH2, JM8.N4, E14 Tg2A.4, and C57BL/6NCr). TMM has continued to successfully complete four comprehensive gene targeting projects, two utilizing the germline-competent ES cell lines on C57BL/6 NCr background, generated within the TMM laboratory, and two projects using the commercially available E14 Tg2A.4 parental cells. This work was performed in support of Drs. R. Sen (NIA), V. Lazarevic (NCI), and J. Farber (NIAID).

Cell Culture

This laboratory has processed tumor cell lines for 29 experiments during the past year in support of the NCI investigators, detailed in the table below:

Investigator	Cell Line	Cell Type
Dr. P. Choyke	MDA MB 361	Breast adenocarcinoma
Dr. P. Choyke	DMS 114	Lung cancer, carcinoma
Dr. P. Steeg	MDA MB 231	Breast adenocarcinoma
Dr. P. Steeg	231 Br eGFP	Breast adenocarcinoma
Dr. P. Steeg	E0771	Breast adenocarcinoma
Dr. P. Steeg	E0771 ffluc eGFP	Breast adenocarcinoma
Dr. P. Steeg	4T1 Luc2	Mammary carcinoma
Dr. L. Wakefield	E0771	Breast adenocarcinoma
Dr. P. Choyke	HT 29	Colorectal adenocarcinoma
Dr. B. Gril	SUM190 BR3	Inflammatory breast carcinoma
Dr. B. Gril	SUM190 BR Luc GFP	Inflammatory breast carcinoma
Dr. P. Choyke	NCI-H69	Small cell lung cancer
Dr. P. Steeg	231 Br Her2	Breast adenocarcinoma
Dr. G. Merlino	Mel114633	Melanoma cell line

Cryopreservation and Assisted Reproduction

Cryopreservation of germplasm (sperm and embryo): Valuable genetically engineered mice (GEM) is an essential service provided to the NIH community and its collaborators by the cryopreservation laboratory. It is critical that these unique mouse models are protected against genetic drift or loss due to unforeseen events, including natural disasters. This laboratory offers an array of services, including assisted reproduction techniques such as in vitro fertilization (IVF), as a means to rescue a given mouse line on the verge of being lost due to breeding deficiencies, or for expansion of a colony to

produce a large cohort for experiments. The cryopreservation laboratory has the expertise to freeze both sperm and embryos, and the modality is recommended to the requesting investigator based on the genetics of the strain, the complexity of its genotype, as well as natural reproductive characteristics of the strain.

Rederivation to specific pathogen-free status (SPF): The cryopreservation laboratory is also responsible for rederivation of mice that require this service if they test positive for pathogens and microorganisms that are excluded from animal facilities on the Frederick or Bethesda campuses.

SIGNIFICANT ACHIEVEMENTS

In the last year, Cryopreservation services were fulfilled for the following institutes: NCI, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institute on Aging (NIA), National Institute of Allergy and Infectious Diseases (NIAID), U.S. Food and Drug Administration (FDA), Animal Production Program, and the NCI Mouse Repository. The Cryopreservation laboratory completed the embryo cryopreservation of 29 strains, which is an increase from the 26 strains completed in FY2015. The program is expected to bank 80 mouse strains via sperm cryopreservation by September 2016, representing an increase from the 60 strains processed in FY2015. Overall, the turnaround time for completion of projects has been reduced all around. Sperm cryopreservation requests are addressed and completed within 3 months from receipt of the mice. The laboratory reconstituted six strains from cryopreserved sperm samples utilizing IVF in comparison with 9 in FY2015, while 6 strains were reconstituted from cryopreserved embryos, a decrease from 22 of such procedures performed the previous year. Live offspring resulting from the reconstitution procedure were shipped to animal facilities designated by the requesting investigators. In addition, cryopreserved materials comprised of 42 shipments of embryos were sent to scientific institutions throughout the world, largely in support of the NCI Mouse Repository.

The Cryopreservation laboratory has rescued one valuable mouse line, and generated large cohorts of male mutant mice for a transgenic mouse model for LEOPARD syndrome in support of a project from NCATS. Production of age-matched transgenic progeny on a monthly basis in support of support of this project has been essential for further characterization of this model. The Cryopreservation laboratory is providing full support to Dr. R. Weigert, (CCR, NCI) to import 20 of his well-established GEMs into the Frederick animal facilities, while simultaneously cryopreserving germplasm from these strains for safe-keeping.

Rederivation by the conventional embryo transfer method was performed for 2 strains, while 11 lines were rederived by IVF for importation into the NCI animal facilities at the Frederick or Bethesda campuses. IVF rederivation requests are completed within 10 weeks from receipt of the males.

Animal Molecular Diagnostic Laboratory

The Animal Molecular Diagnostics Laboratory (AMDL) performs nucleic acid-based diagnostics using polymerase chain reaction (PCR) technology as part of the LASP animal health surveillance program. AMDL also serves as the genetic quality control laboratory for the NCI Mouse Repository and the LASP cryopreservation program. In addition, the laboratory conducts mycoplasma assays for NIH investigators.

SIGNIFICANT ACHIEVEMENTS

- During this reporting period, 25,987 diagnostic samples, tail biopsy, and cell culture samples were processed and tested. AMDL performed 11,226 molecular diagnostic tests for animal health monitoring, 12,636 PCR assays for 738 cell line Molecular Testing of Biological Materials (MTBM) tests, 21,236 mouse genotyping and 653 mycoplasma assays for the NCI Mouse Repository and NCI/NIH investigators.
- AMDL developed and launched the in-house Fur Mite PCR assay as part of routine health monitoring. The assay is more sensitive and has been used in assessing the status of mice transferred into animal facilities from the quarantine room where the fur mite ova was found as described in the previous AHDL section.
- AMDL continue to work in sync with AHDL to provide molecular testing on the environmental monitoring of the isolators and supplies for the Germ Free Project. The universal 16S RNA gene PCR assay provides an essential tool to assess the sterility status of these isolators and allow early detection of any potential breach of Germ Free status of these isolators.
- Completed development and implemented the PCR assay for segmented filamentous bacteria (SFB) to support the monitoring of the source animals for Dr. Lazarevic's Experimental Autoimmune Encephalomyelitis (EAE) projects housed in the Building 10/Tower facility.
- Completed development and implemented the PCR assay for *Stenotrophomonas Maltophilia* to support specific health monitoring of the isolator for NCI-DTP program.

Speed Congenics Service

The LASP Speed Congenics Service, including genetic testing and colony management, derives congenic strains of mice for NIH intramural investigators by marker-assisted backcrossing. Through analysis of polymorphic microsatellite markers and selection of optimal breeders, congenic mice can usually be obtained in 12 months, whereas they may take as long as 2.5 years to produce by conventional backcrossing.

SIGNIFICANT ACHIEVEMENTS

Five new projects have been initiated. Six genomic scans were performed in support of four investigators. The supported projects are detailed in the table below.

Principal Investigator	Affiliation	Number of Projects	Status
Dr. M. Christine Hollander	NCI, CCR	3	Genome scan
Dr. Yun Ji	NCI, CCR	1	Genome scan
Dr. Vanesa Sanchez	NCI, CCR	1	Genome scan
Dr. Jyoti Sen	NIA	1	Genome scan

Small Animal Imaging Program

SAIP provides a state-of-the-art in vivo imaging facility for studying intact biological systems for researchers at NCI's CCR, Division of Cancer Treatment and Diagnosis (DCTD), Center for Strategic Scientific Initiatives (CSSI), and the NCI at Frederick Office of the Directorate (OD) and Office of Scientific Operations (OSO).

The function of SAIP is to assist investigators in developing mouse models, determining new targets for early detection and therapy, and testing potential new therapies without sacrificing the animal. Another core function of SAIP is to assist the CSSI Nanotechnology Characterization Laboratory (NCL) to analyze nanoplatforms. In addition to these core functions, SAIP's multifaceted mission is to assist the DCTD initiatives in developing standards in small animal imaging and integrate imaging into drug development.

The noninvasive in vivo imaging facility (greater than 7,500 square feet) incorporates a clinical 3T magnetic resonance imaging (MRI) utilizing specially designed rodent coils, nuclear scanners (positron emission tomography/computed tomography [PET/CT] and single-photon emission computed tomography/computed tomography [SPECT/CT]), optical scanners (bioluminescence, fluorescence, and tomographic fluorescence), high frequency (40 MHz) rodent ultrasound and photoacoustic scanners, a gamma-well counter, and several high-end image processing workstations.

SIGNIFICANT ACHIEVEMENTS

New Imaging Techniques

New procedures developed by SAIP include: 1) collaborated with Image Visualization Group/ABCC/DSITP for the conversion of VisualSonics Ultrasound native format images to 3D Ultrasound DICOM format (radiology standard image format), 2) enhanced the photoacoustic oxygen saturation protocol, 3) implemented a multi (4)-MRI volume coil to conduct simultaneous imaging on four mice to increase throughput and 4) developed and implemented intracardiac and intrahepatic ultrasound image guided injections.

Patient Derived Xenograft Models (DCTD)

The SAIP conducted several studies for the Cancer Imaging Program (DCTD) to characterize metastasis in PDX mouse models; including MRI (non-contrast and contrast-Eovist), Ultrasound (3D volumes), and [^{18}F]FDG PET/CT for glucose metabolism. The SAIP also conducted drug challenge studies to understand the change in the uptake of an imaging probe due to a therapeutic drug.

Molecular Imaging Probe Characterization (CCR-CRADA)

The SAIP conducted two large imaging probe characterization (validation PET/CT imaging, nuclear biodistribution, and internal radiation dosimetry) studies utilizing ^{89}Zr-tagged probes for the Molecular Imaging Program (MIP)/CCR as part of a CRADA with MedImmune. These studies are conducted in collaboration with SAIP, LASP technical support, LASP facility staff, PHL, MIP staff, and the Imaging Probe Development Center (NIH core).

SAIP conducted 68 imaging projects in support of the following programs/divisions:

Division/Program	Projects
Center for Cancer Research	
Cancer and Inflammation Program	2
Cell Biology/Experimental Immunology Branch	2
Center for Advanced Preclinical Research	7
Chemical Biology Laboratory	2
Cytokines and Immunity Section/Cancer and Inflammation	1
Human Retrovirus Section\Vaccine Branch	2
Laboratory of Cancer Biology and Genetics	1
Laboratory of Cell and Developmental Signaling	2
Molecular Imaging Program	1
Oncogenomics Section/Genetics Branch	1
Thoracic and Gastrointestinal Oncology Branch	6
Urologic Oncology Branch	5
Women's Malignancies Branch	4
Center for Cancer Research (CRADA)	
Center for Advanced Preclinical Research (AstraZeneca)	1
Center for Advanced Preclinical Research (SolaranRx)	2
Molecular Imaging Program (MedImmune)	2
Center for Strategic Scientific Initiatives	
Nanotechnology Characterization Laboratory	2
Division of Cancer Therapy and Diagnosis	
Cancer Imaging Program (PDX-Models)	22
Leidos Biomedical Research, Inc. (TSA)	
Center for Advanced Preclinical Research (Abbvie)	3

Infrastructure and Operational Enhancements

Small Animal Imaging DICOM committee (NEMA/DICOM: WG30)

The Digital Imaging and Communications in Medicine (DICOM) Small Animal Imaging Working Group (WG30) developed, and approved by the international committee, a standard image format for small animal imaging incorporating the image data acquisition and numerous other parameters (physiological waveforms, animal model, injection route, and the labeling and location of each mouse in a multi-mouse acquisition). This standard integrates numerous parameters to enhance the capability to perform co-clinical studies. The WG30 collaboration was composed of the DICOM international committee, National Cancer Informatics Program/CBIIT, Division of Cancer Biology, Cancer Imaging Program/DCTD, and external investigators (academic and industry). Dr. J. Kalen (co-chair).

HyperPolarizer Project

The Division of Cancer Therapy and Diagnosis/NCI requested SAIP to provide a cost analysis to implement and operate a ^{13}C hyperpolarizer with associated equipment for studying metabolism utilizing MRI nuclear spectroscopy techniques ($911K). DCTD approved the project; the MRI suite was renovated for the hyperpolarizer and equipment ordered.

Pathology/Histotechnology Laboratory

The Pathology/Histotechnology Laboratory (PHL) provides research specialized support for the FNLCR/NIH including animal health monitoring, disease model expertise, phenotyping of genetically engineered mice, pre-clinical toxicity studies, and support for molecular profiling of tumors. Interactions with investigators are a constant emphasis. A Veterinary Pathology Section (VPS) provides experimental design consulting, development of necropsy and trim protocols, and comprehensive pathology evaluation. The full-service Histotechnology Laboratory (HL) additionally offers powerful tools for rodent experimental studies including blood chemistry, hematology, complex necropsy support, optimal cutting temperature (OCT) (frozen) and paraffin block production, microtomy, cryotomy, and a full range of histological stains. The HL also offers a range of molecular histotechnology services including Western blotting, immunohistochemistry (IHC) for more than 600 validated targets, chromogenic in situ hybridization (CISH), RNAScope™ CISH, and nucleic acid isolation from human research specimens via hand/macro-dissection, laser capture microdissection (LCM), and laser cutting (LC). PHL also offers Leica bright field and fluorescent digital whole slide scanning with image annotation capabilities and digital image analyses options, and has banked a wide range of reagents used to facilitate research and develop novel assays.

Infrastructure and Operational Enhancements

RNAScope ISH Assay Support

PHL completed evaluation of a new RNA in situ hybridization (ISH) assay that is greatly improved over prior offerings and is now our recommended procedure.

MMI CellCut Procurement

PHL replaced a nonfunctional infrared laser-cutting instrument with a new automated CellCut laser-cutting microscope, which has increased the efficiency of our laser capture microdissection (LCM) services.

Nanodrop One Procurement

PHL has purchased a new Nanodrop One machine which can identify contaminates and corrects absorbance readings for more accurate concentrations. Due to its Wi-Fi capability, resulting in a smaller footprint, it can be used anywhere within the laboratory.

SIGNIFICANT ACHIEVEMENTS

- **VPS**
  - PHL filled both open Pathologist positions: Center for Advanced Preclinical Research (CAPR) and PHL.
  - Performed approximately 14,000 slide equivalents (slides, plus professional time).
- **HL**
  - Darlene Green, research associate, retired after 36 years of service for the FNLCR.
  - PHL filled five replacement positions within the laboratory.
- **Laboratory of Pathology, Pathogenetics Unit, Advanced Technology Center, CCR, NCI.** PHL developed a method for tissue preservation that combines a novel tissue fixative with commercially available molecular stabilizers that yield RNA quality equal to that of unfixed frozen tissue, the current gold standard.
- **Novel IHC Method.** PHL developed a novel method for the dual IHC detection of mouse CD4 and CD8a in paraffin-embedded tissues. Previously, this had only been possible on acetone-fixed frozen tissue sections.
- **National Clinical Target Validation Laboratory and Pharmacodynamics Assay Section.** Expanded support that includes H&E, fluorescent IHC staining and image capture on biopsies from human and canine patients on research clinical trials.
- **Drs. Doroshow and Hollingshead (NCI, DCTD, DTP, BTB).** Completed IHC evaluation of Duox2 IHC on multiple human pancreatic cancer tissue microarrays (TMAs).

- **Drs. Howard Young and Julio Valencia (NCI/CCR/CIP).** Continued support for studying the role of interferon gamma (IFN-γ) in oncogenesis, metastasis, and drug resistance in melanoma.
- **Dr. Peter Johnson (NCI/CCR/MCGP).** Provided support for his studies involving effects of Cebp-β mutations on cancer development and progression.
- **Dr. Vanja Lazarevic.** Working together with Bethesda lab animal veterinarians and animal facility staff, provided histopathology support to investigate reason for recent failure of studies involving EAE mouse models.
- **Nanotechnology Characterization Lab (NCI/OD).** Continued to provide necropsy and histopathology support for several efficacy, toxicity, toxicokinetics, and imaging studies.
- **Drs. Piyush Agarwal (NCI/CCR/UOB), Christina Annunziata (NCI/CCR/WMB), Peter Choyke (NCI/CCR/MIP), Shyam Sharan (NCI/CCR/MCGP/GCSS) and over 30 additional principal investigators.** Evaluated mouse and human IHC, including TMAs, in addition to H&E-stained sections.
- **Drs. Raffit Hassan (NCI/CCR/TGOB).** PHL completed a LCM mesothelioma RNA and DNA sequencing project.
- **Dr. Mary Barcus (NCI/DCTD/CDP/BBRB).** PHL completed a LCM stomach and pancreas RNA sequencing project.

High-Throughput Animal Genotyping Laboratory

High-Throughput Animal Genotyping Laboratory (HTAGL) provides genotyping in high-throughput format using ABI TaqMan technology (as formerly offered by the Laboratory of Molecular Technology) and end point PCR using the LabChip as the detection platform. Enhanced workflows in conjunction with a Laboratory Information Management System (LIM) are used to automate the entire genotyping process from sample submission to final reports.

SIGNIFICANT ACHIEVEMENTS

- During this reporting period, HTAGL has processed approximately 12,260 samples and determined 50,000 genotypes that an approximately 25 percent increase from the number in 2015. HTAGL continues to largely support NCI's CAPR, but continues to add new NCI investigators into the supporting list.
- Data Management Services complete an improved LIM that supports additional assay formats, clients, reports, and billing.

Support Provided by the Data Science and Information Technology Program

Advanced Biomedical Computing Center

The Advanced Biomedical Computing Center (ABCC) supports scientific research at the Frederick National Laboratory for Cancer Research (FNLCR), NCI at Frederick, NCI in Bethesda, NIH, and other federal agencies through the Economy Act. The ABCC provides bioinformatics, mathematical simulation and modeling, image analysis and visualization, nanoinformatics, and proteomic analysis expertise to these communities. In addition, it provides core infrastructure and data integration support for scientific projects through database maintenance and development, software maintenance, and scientific web application development.

ABCC support is provided through a variety of mechanisms, including provisioning of bioinformatics applications and databases; help with experimental design; data and results interpretation through consultation, collaboration, and training; technology development and enhancement; outreach to local academic communities and national resources provided at other national labs; and serving as an interface with commercial and nonprofit organizations.

The Imaging and Visualization Group

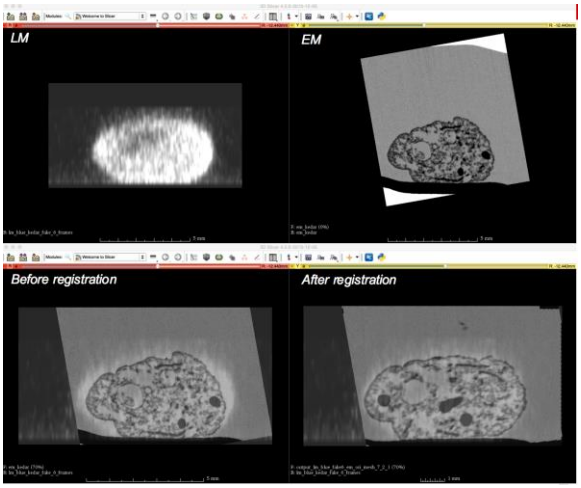
The Imaging and Visualization Group (IVG) supports and accelerates basic research by developing and implementing technologies in image analysis; scientific visualization; IT and software infrastructure; and services to facilitate data access, collaboration, and reuse; reduce duplicate efforts; and automate labor intensive workflows. Highlights for this year include 3-D electron microscopy (EM)/light microscopy (LM) image volume registration and web-based real-time visualization.

3-D EM/LM Image Volume Registration

The NCI Center for Molecular Microscopy (CMM) applies advanced technologies for 3-D electron microscopy to solve problems of fundamental interest in cancer and HIV/AIDS biology. At the CMM, a central area of focus is accurate 3-D correlative light and electron microscopic (3-DCLEM) imaging of cells and tissue. In 3-DCLEM, 3-D images from optical microscopy are merged with 3-D images from electron microscopy to images that are more feature rich. Merging the images is challenging due to the different resolutions, modes of distortion, and unequal imaging planes between optical and electron microscopy images. Therefore, it is important to image the same area of interest by LM and EM and also ensure that the software algorithms correct the alignment and overlay these 3-D image volumes with high fidelity.

The 3-D registration work aims to solve this problem by focusing on robust alignment of 3-D LM and EM image volumes without resorting to fiducial landmarks, such as heavy metal particles that distort and alter the samples.

IVG had developed an automatic pipeline with deformable registration using a technique called Mutual Information. The pipeline is able to accurately register uniformly stained optical and electron microscopy image volumes. The pipeline will be tested by CMM scientists and readied for production once the initial testing and refinement is finished. This will allow NCI scientists, as well as others who wish to install the software at their local sites, to integrate optical and electron images to gain greater insight into structural features of cells and tissues for biomedical research.

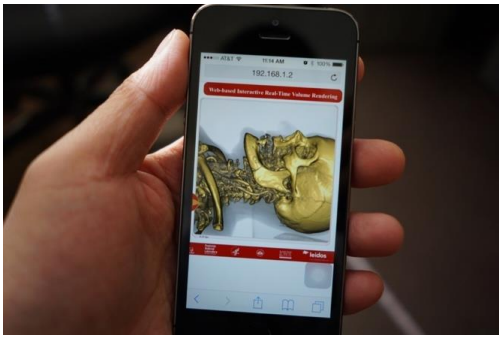


Registration of the dictyostelium worm EM-LM dataset. Top-left: cross-section view of LM image volume (slice thickness adjusted). Top-right: cross-section view of the corresponding EM image volume. Bottom-left: cross-section view of manually pre-aligned LM-EM image volumes. Bottom-right: cross-section view of automatically registered LM-EM image volumes. The result shows major features on LM and EM images are accurately aligned in 3-D space. Image data provided by CMM.

Web-Based Real-Time Visualization: Ray-Casting 3-D Biomedical Image Volume Rendering System

The ABCC IVG is actively developing a web-based image visualization system that provides state-of-the-art real-time volumetric visualization on cross-disciplinary image data. IVG collaborates with the University of Central Florida (UCF) to perform the development work and will deploy the system to the FNLCR community.

This work is aimed at streamlining visualization workflows for desktop computers, laptops, and mobile devices such as phones and tablets. The software allows investigators to render large size volumes not suitable for regular desktop computers without downloading the images, thus providing greater ability to collaborate, preserve image provenance, retain the images in a secure and trusted environment, scale as image sizes and quantities increase, decrease maintenance costs by supporting a single instance and version, and lower the barrier to image visualization for scientists, researchers, and clinicians. The tool can also be integrated with picture archiving and communication systems (PACS) and other image archives to create a unified image query and visualization experience. This work generated considerable interest when it was presented at the NIST BioImage Informatics Conference 2015. Groups such as the Allen Brain Institute, Harvard, Kitware and 3Scan (a startup company based in San Francisco) have expressed interest in this technology through collaboration and support letters. Other vendors, including GE and Autodesk, had also expressed strong interest in the development and adoption of this tool. This interest by external organizations provides confirmation that the IVG web-based ray-casting volume-rendering tool is much needed and should find wide adoption when the first version is released.



Picture showing the IVG-developed ray-casting rendering system running on an iPhone 5s device. The displayed image is rendered at real-time from user-selected image volume. Users are able to rotate and pan to examine the image volume from different angles and positions. The system allows investigators to access state-of-the-art volume visualization technologies on both desktop workstations and mobile devices.

Text Mining for Nanomaterials Data Extraction. Nanoparticle Data Correlation Tools. Imaging Techniques for Nanomaterials Analyses—High-Dynamic-Range Imaging for Nanoparticle Tracking Analysis and Nanoparticle Genotoxicity. Electron Microscopy Nanoparticle Reconstruction

Engineered nanomaterials are a promising new platform for exploring new strategies in cancer treatment and detection. Predicting the emerging properties of modified nanoparticles remains a challenge, and the possible emergence of unexpected side effects (toxicity) is a concern. Mining extensive data collections to explore hidden relationships to improve the nanomaterial or avoid potentially dangerous combinations is an area of active exploration and development that shows considerable promise. Gathering the required collections of datasets for nanoparticles, however, is a difficult challenge. Nanomaterial characterization for biomedical applications is the ultimate multidisciplinary effort involving synthetic chemists, physical chemists, biologists, and physicians, resulting in very dissimilar types of publications and reports ranging from technical descriptions to clinical trials, scientific publications, and patents. The ABCC worked with the Nano Materials Registry, the premier

repository for engineered nanomaterials, to assess and develop tools for text analytics to aid in the mining of scientific literature, including the use of fast approaches (micro-rules), ontology-free text analysis, and rule and data mining. These developments have increased the ability to scan literature on nanomaterials by two orders of magnitude. Combined with a much-enhanced ability to mine data from literature, the pipelines facilitate powerful implementations of data exploration tools, including set logic and graph analysis. Nanomaterials are extremely complex in their variety and composition. The use of predictive tools is, therefore, an essential part of the nanomaterial optimization pipeline. Yet, quantitative structure activity relationships for nanomaterials (Nano-QSAR) are hindered by the lack of data from comparable experimental settings and the inherent properties of the materials (i.e., polydispersion and polymorphism). To address parts of these challenges, the ABCC has created specialized tools for the simulation of dendrimers, metal, and metal oxide nanoparticles as a first step in exploring the fundamental properties that lead to the experimental challenges. To realistically survey the property space and compare with observed properties, the tools have been built with unique characteristics like their high speed of execution, which is necessary due to the very large number of scaffolds that need to be computed to create a statistically meaningful ensemble that faithfully represents the nanomaterial complexity. Experimental data to correlate with the 3-D structure properties can be obtained using imaging methods and simulation.

To integrate imaging information in a semi-automated manner, the ABCC has developed multiple optical imaging methods to characterize nanomaterial genotoxicity based on the micronuclei assay and single-molecule-level physicochemical characterization of nanoparticles based on nanoparticle tracking analysis (NTA). NTA platforms rely on a laser illumination system coupled with an optical magnification path, allowing the rapid and accurate tracking of single-particle positions in liquid suspensions. Traditionally, the particle tracking information is used to estimate the particle hydrodynamic radii and other properties of interest. However, more information may be present in the signal if appropriate contrast and signal-to-noise enhancements can be realized. We utilized advances in high dynamic range (HDR) photography as a relatively inexpensive way to obtain additional information (i.e., shape signatures) from NTA trajectories. The physicochemical characteristics of the materials obtained at the single particle level can then be correlated with biological markers. HDR is also used to improve micronuclei count in cell lines exposed to nanomaterials and is being used to develop pipelines to create extensive image libraries for training new machine learning algorithms such as Deep Learning. These methods are also applicable to other imaging areas such as EM image pre-processing and has resulted in the structure of the first nanoparticle obtained using low-voltage EM (publication pending). In short, the ABCC has developed novel techniques and implemented integrative approaches to the difficult problem of in-silico characterization of nanomaterials to aid researcher in this area speed up their quest for more powerful cancer treatments.

Core Infrastructure and Systems Biology Group

The Core Infrastructure and Systems Biology (CISB) group strives to streamline biological infrastructure and provide innovative solutions for the NCI/NIH community to access and use biological information spread across different sources and formats. In addition to providing the biological database and bioinformatics application support, CISB has continued to develop and improve the infrastructure applications.

Biological DataBase Network

In the past year, the application was in an Operations and Maintenance (O&M) phase, and there have been no new releases for the biological DataBase network (bioDBnet). The automatic updates on the backend databases supporting the application have been running without any major glitches, and CISB continues to provide support for user queries and resolve any bugs associated with the existing features in bioDBnet.

The application continues to see increasing adoption by the research community. The number of users have increased from 10,329 (FY2015) to 13,168 (FY2016) within the same date range, October 1 to July 20.

Annotation, Visualization, and Impact Analysis

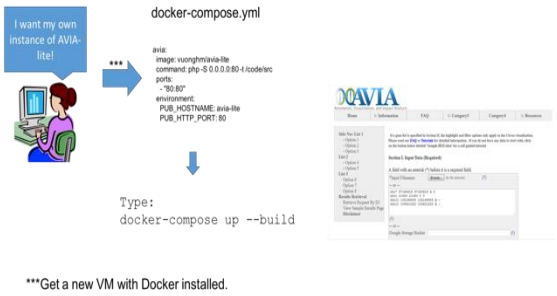
The application for annotation, visualization, and impact analysis (AVIA) has been added to the sequencing pipelines developed by the Collaborative Bioinformatics Resource (CCBR) supporting the sequencing efforts in the Center for Cancer Research (CCR). To provide this service, CISB worked closely with the CCR bioinformatics core, as well as the administrators for the Biowulf batch system in Bethesda. CISB helped resolve issues with command line access and enable usage across the NCI and Frederick firewalls. In addition, CISB built new web pages to provide status updates on the annotation pipelines. A joint presentation by CISB and CCBR on the Exome-Seq pipeline and AVIA integration into the Exome-Seq pipeline was given at the Bioinformatics Training and Education Program (BTEP) workshop.

Based on user feedback, a lack of annotation resources for mouse sequence variants has been identified, and CISB has recently added mouse annotations to the core functionality of AVIA.

Cloud-Ready through a Docker Instance for AVIA

As a pilot effort to create new Docker instances and share infrastructure applications created by the CISB group, a lighter version of AVIA, named AVIA-lite, was created. AVIA-lite does not have the batch computing dependencies of AVIA and has a limited set of variant annotations.

The AVIA-lite application and all its dependencies were then containerized using Docker. Extensive documentation and step-by-step tutorials on installing the Docker version of AVIA-lite were shared with the broader group, and an independent installation of the instance was done to check for any application bugs or missing documentation.



Workflow showing the installation steps for AVIA-lite using Docker.

Custom Protein Visualization Search Tool (CyPRUS)

- In FY2015, CISB developed the CyPRUS application to visualize protein features and also overlay any custom annotations, such as variants, onto the 2-D image. This enabled the researchers to visualize all the features that might or might not be impacted by specific protein features and domains. CyPRUS also enabled the same visualization on all isoforms of the protein, thereby providing valuable information for researchers interested in noncanonical forms. In FY2016, based on user input, we have added additional features to CyPRUS that would enable users to visualize the features in all available protein orthologs. CyPRUS uses the dbOrtho feature in bioDBnet to get all the homolog proteins and then displays all available UniProt features for each of the orthologs.

Merged screenshots from CyPRUS displaying the protein features in the canonical form of ARAF, Isoform-2 of ARAF, and the ARAF ortholog in Zebra Fish.

Scientific Web Programming Group

The ABCC's scientific web-application programming group (SWPG) enables and supports NCI science by providing innovative web application and tool development to assist groups and researchers with managing and tracking data and interacting with data and scientific applications through web interfaces.

The site supports FNLCR as well as Data Science and Information Technology Program (DSITP) and its groups by offering applications and tools to assist in data management. Supporting an open-source content management system, WordPress allows authorized users to manage site structure, content, and services. Users have the ability to manage their content and determine access requirements to their content, but NIH Active Directory authentication is required prior to allowing viewing privileges, and specific pages to are restricted to authorized users only.

The ABCC has continued to support FNLCR groups through the Leidos Biomedical Research (Leidos Biomed) publications application (https://publications-abcc.ncifcrf.gov). This enterprise-level application supports Leidos Biomed/FNLCR by recording and tracking all publications, manuscripts, inventions, and journals created by FNLCR employees for reporting to FNLCR and NCI management. Continued user support and features and tools enhancement are the majority of current efforts for this operational project, and the ABCC has provided continued support by participating in numerous presentations and tutorials throughout the year. Recent additions include utilities that allow editors to run reports that focus on incomplete and orphaned records to maintain quality data. In addition, a new report tool allows editors to segregate publications by journal name, a useful tool when management wants to know which journals contain FNLCR publications. The publications application currently supports 128 active users across 44 groups with 838 authors named within over 4,000 publications.

Project Tracker: ABCC/SWPG developers continue to support the Office of Scientific Operations (OSO), NCI at Frederick, while building a project management tool that will assist the ABCC with managing and tracking projects and tasks and assist NCI in review, approval, management, and prioritization of the technology development portfolio, as well as other ABCC projects. Recent accomplishments include reporting features that allow external users access to project information such as status updates, overall progress, and project histories. Also added was a profile section for all users, which allows users to flag their skillsets and add a biography for other managers and users. A search feature gives users the ability to find each other based on skillset. This tool is helpful when users are in need of assistance or collaboration while working on day-to-day tasks.

Simulation, Analysis, Mathematics, and Modeling Group

The simulation, analysis, and mathematical modeling (SAMM) group uses mathematical and statistical analyses, high-level quantum chemical calculations, protein structure modeling, protein structures' interactions with drugs, and computational simulations to model the initiation, progression, and putative treatment of cancer and HIV/AIDS.

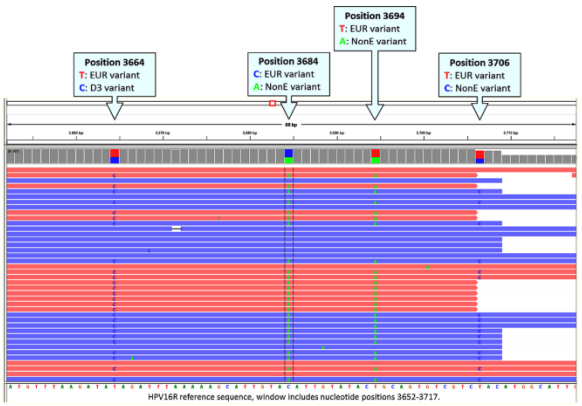
Selected Activities

Modeling the Functional Impact of Disease Pathway Associated Genes

Next-generation whole-genome and whole-exome sequencing of cancer tissues has consistently revealed the occurrence of many high-risk mutations, especially in disease pathway–associated genes. In many cases, these mutations can lead to ligand independent auto-activation or a gain-of-function. The urgent goal of many cancer research projects is to identify susceptibility genes that might be linked to the concerned cancer type and also to discover predisposing mutations, if any, using data from disease-prone patients. Since the 3-D structural fold of the protein holds the key to its function, supplementing the static/dynamic features of structural data with the sequence information (database search, alignment, homologous genes, etc.) often reveals key information about the role of the concerned gene/protein in a disease pathway. The following two SAMM collaborative projects are examples of such an approach.

Deep Sequencing of Human Papillomaviruses-16 (HPV16) Genomes: Discovery of Novel SNPs and Large Scale Deletions Indicative of Viral Integration

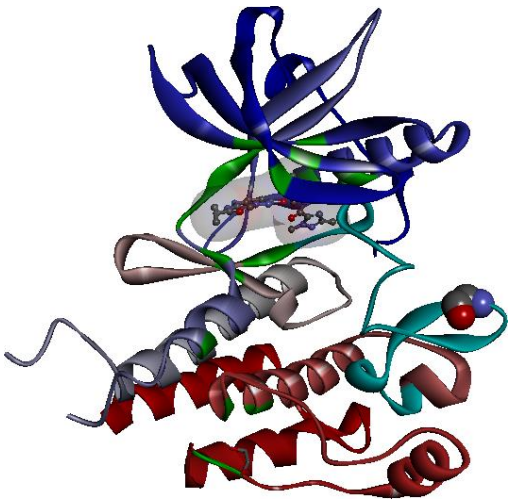
Human papillomavirus (HPV) is a name given to indicate 150 related viruses. HPVs can cause a variety of cancers, the most common of which is cervical cancer. According to the Centers for Disease Control and Prevention (CDC), HPV causes almost 30,700 new cases of cancer each year, and the most worrisome part is there is no way to predict whether or not the people infected with HPV will eventually get cancer. Better screening procedures and markers are needed for the different types of HPV. In this study, Drs. Mike Cullen, Lisa Mirabello, and colleagues from DCEG developed a high-throughput NGS technique to identify novel single nucleotide polymorphisms (SNPs) that result in structural variations in HPV16 that might be able to explain viral infections that assist in cancer formation. SAMM scientists were invited to join this team and participated in the analysis of HPV variants and their impact to the viral activity using a variety of impact analysis methods. This study has been published in the official journal of the International Papillomavirus Society, *Papillomavirus Research* (1:3-11, 2015). The study was recently hand-picked as one of the Papillomavirus Research Editors' Choice articles. SAMM scientists continue to collaborate with Drs. Mirabello and Cullen in their ongoing HPV projects.



Gleaning of HPV16 variant lineage co-infection using sequence alignment. EUR: European. NonE: non-European.

Whole-Genome Sequencing of High-Risk Hodgkin's Lymphoma Families and Structural Bioinformatics-Based Analysis Identifies a Predisposing Mutation in KDR Gene

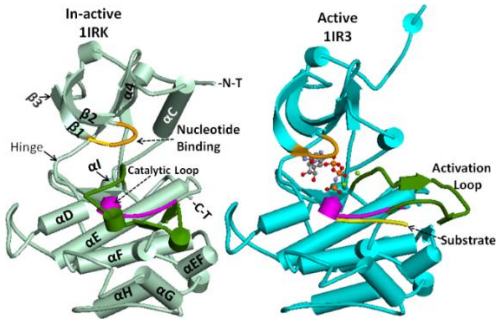
Hodgkin's Lymphoma (HL) is a blood cancer that begins in white blood cells and eventually targets the immune system. The American Cancer Society estimates that there will be about 1,120 fatalities (480 females and 640 males) in 2016. Using exome sequencing of 65 HL families, Dr. Lynn R. Goldin (NCI, Division of Cancer Epidemiology and Genetics [DCEG]) and her collaborators had identified a rare nonsynonymous c.3193G>A (p.A1065T) germline mutation on the KDR gene that appears to cause a predisposition for HL. Experimental studies show that the modified residue, A1065, is part of the kinase activation loop, hence the newly acquired residue (THR; A1065T) has the potential to promote autophosphorylation and probably provide a gain-of-function for the KDR protein product, VEGFR2. This study has the honor of the largest sequenced cohort of HL families to date. SAMM scientists, along with Dr. Goldin and collaborators, assessed the functional impact of the mutation using a novel sequence/structure-based approach that included the genomic splicing patterns and the sequence/structure-based analyses. This work was recently published in the high-impact journal, *Haematologica*, 101(7):853-60, (2016).



Experimental 3-D structure (PDB ID: 3VO3) of an inhibitor drug–bound kinase domain of VEGFR2. The location of the newly identified predisposing HL mutation, p.A1065, on the activation loop is shown in a solid spheres (CPK) style.

Modeling Cancer Drug Inhibition Using Protein-Ligand Docking

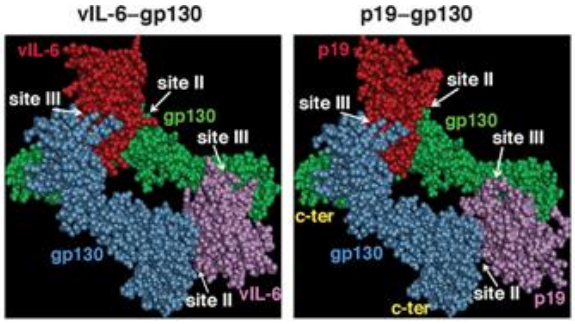
Cancer often arises from uncontrolled proliferation of cell-signaling events. Protein kinases have been identified to be the central players and have thus become the main therapeutic targets of many cancer drugs. In this study, SAMM scientists used the protein-ligand docking technique to show how the kinase receptor active-site 3-D features play a key role in inhibition and how this knowledge can in turn be used to design family-specific inhibitors. They also demonstrated that, by combining biological pathway information with a structure-focused model, it is possible to design inhibitors with fewer side effects. The study had been published in the journal that is dedicated for molecular modeling and computational chemistry, *J Mol Graph Model*, 57:36-48 (2015).



Comparative view of kinase active site that highlight the differences between the active and inactive conformations. Experimental structures of insulin kinase receptors were chosen for the comparison.

Application of Comparative Modeling and Structural Bioinformatics Approaches to Understand the Biology of Inflammation

Dr. Giovanna Tosato (Lab of Cellular Oncology, CCR, NCI) led a study in search of proinflammatory signals within endothelial cells that promote diseases such as giant-cell arteritis (GCA). The results from this study showed that the GCA patients had endothelial proinflammatory peptide p19 (IL-23p19) associated with a glycoprotein (gp130). This information, along with the lack of IL-12p40 chain, indicated that p19 might be a potential therapeutic target for GCA disease drug discovery efforts. SAMM scientists participated in the study and used the concepts of homologous protein folds to predict the potential binding mode of p19 and gp130. The experimental structural template complex of vIL6-gp130 (PDB ID 1I1R) was used as a structural template to predict the potential binding mode of human p19-gp130. This high-impact work was published recently in the premier journal of cell signaling in physiology and disease, *Sci Signal*, 9(419) 1-10 (2016).

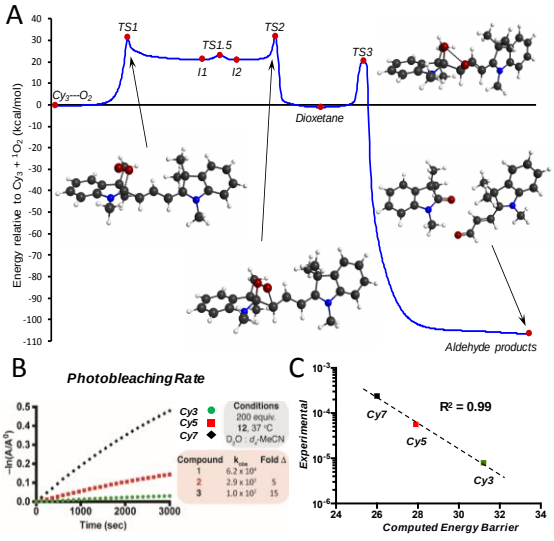


Modeling the binding interactions of p19-gp130 exploiting the structural similarity of the experimental vIL6-gp130 tetrameric complex (PDB ID: 1I1R).

Quantum Chemical Tuning of Chemical Biology Tools for Cancer Therapy and Diagnostics

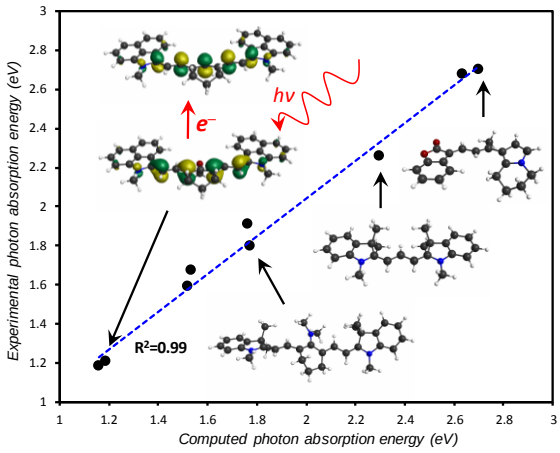
The SAMM group is developing high-level quantum chemistry methods and using them to assist NCI researchers in the design of chemical biology tools for use in diagnostic and therapeutic applications. These quantum chemistry approaches allow for the expedient computational study of a range of hypothetical molecules to determine if any of them have the desired chemical properties, thus conserving significant resources associated with intricate and complex experiments. In this way, a range of potential candidate molecules can be pruned to include only those showing the most potential. In one study, the Chemical Biology Laboratory (CBL) is developing next-generation optical tools for therapeutic applications that include light-activated drug delivery, molecular probes, and cellular imaging. These processes are based on the fragmentation of cyanines by reactive oxygen species (ROS). One goal is to generate a range of systems with differing reaction rates so that drugs may be dosed variably vs. imaging applications that require slow, extended release. We have used quantum chemistry

techniques to map, for the first time, the complete reaction profile of this chemical process, and identified precisely the energy barriers (or bottlenecks) that must be overcome. Furthermore, we have demonstrated that our computed barriers correlate almost exactly with experimentally-measured reaction rates for a number of model systems. The results show that we are able to reliably predict reaction rates, thus allowing for the computational discovery of novel molecular systems having tailored properties. A manuscript describing this work will be submitted for publication to the *J Am Chem Soc*.



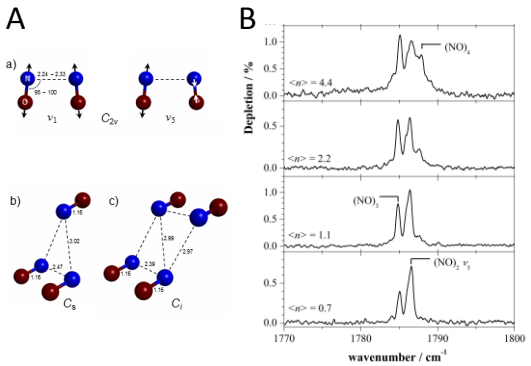
Panel A) Full energy profile and mechanism for the photobleaching reaction of the Cy3 model system as determined from high-level quantum chemistry calculations; panel B) experimentally observed photobleaching rates of Cy3, Cy5, and Cy7; panel C) excellent agreement is obtained between computed energy barriers vs. experimentally measured photobleaching reaction rates.

One fascinating aspect of the chemical processes described above is that they only occur upon absorption of light waves, whereby each unique molecule has its own signature wavelength (or energy) that starts the reaction. This means that drug-delivery can be initiated only by irradiation of the correct "color" of light, thus providing almost complete control over where, when, and how a therapeutic agent is activated. The CBL is aiming to design a spectrum of molecular tools that are activated with varying light wavelengths for tuned delivery, e.g., near-infrared activation allows for significant tissue penetration. We have developed quantum chemistry methods that calculate the light activation energy of essentially any molecule and shown that, for a wide range of model systems, our computed data match those of experimental measurements (see figure below). Furthermore, we are providing guidance to CBL on which molecular systems are most likely to have the desired light-activation properties they seek.



Excellent agreement is obtained between theoretically computed and experimentally measured photon absorption energies for a diverse range of light-activated cyanine systems; some molecular structures are illustrated together with the actual computed electron orbitals involved in the electron excitation of the Cy7-OMe-C6H6-Na system upon light irradiation (above trend line).

High-level quantum chemistry methods have also been used to successfully model nitric-oxide releasing prodrugs, and it was serendipitously found that nitric oxide can condense to form larger clusters than just dimers. Two years after our publication of the prediction of high-order clusters, researchers at the University of Southern California (USC) observed experimentally what they thought might be NO trimers and tetramers and reached out to us to confirm their findings. We computed spectral properties of the clusters and concluded that the USC group had indeed likely evidenced their existence. An article describing this work has been published in *J Phys Chem A*.



A) Computed structures of a) NO dimer (two vibrational modes shown), b) NO trimer, and c) NO tetramer; B) experimental infrared spectra in range of ν_5 band showing NO trimer and tetramer signals.

The Somatic Autosomal Mutation Matrix in Cancer Genomes

DNA damage in somatic cells originates from both environmental and endogenous sources, giving rise to mutations through multiple mechanisms. When these mutations affect the function of critical genes, cancer may ensue. Although identifying genomic subsets of mutated

genes may inform therapeutic options, a systematic survey of tumor mutational spectra is required to improve our understanding of the underlying mechanisms of mutagenesis involved in cancer etiology. The SAMM group has recently identified a tissue-specific mutation pattern in melanomas. This mutation pattern was also identified in healthy eyelid tissue using the data from Dr. Iñigo Martincorena and coworkers (*Science*, 348(2015)880-886). This work shows that mutation processes occur in healthy tissue, and when the right combination of genes is mutated, tumorigenesis may occur.

Germline Mutations in Cancer Driver Genes

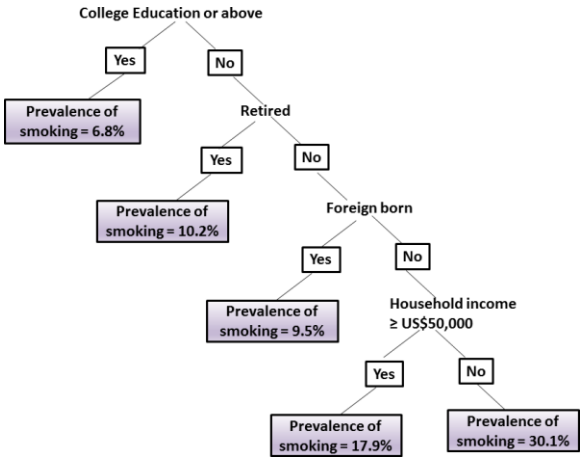
The current model of tumor formation is evolving to include the possibility that several cancer driver genes are already mutated at birth. To investigate this, the germline mutations in 1,094 individuals from the 1000 Genomes Project were examined to determine if deleterious mutations were present in any of the 125 cancer driver genes identified by Dr. Burt Vogelstein and coworkers (*Science* 339(2013)1546-1558). 102 of these 125 genes contained a deleterious mutation in at least one individual. Ten genes were mutated in only a single individual, while ERBB2, FLT3, RNF43, and MLL3 were deleteriously mutated in 814, 857, 984, and 1,080 individuals, respectively. All individuals had a deleterious mutation in at least three cancer driver genes, and one individual had mutations in 18 genes. Future investigations will examine the pathways affected by these mutations.

Mathematical and Statistical Support for NCI/NIH Research

Mathematical and statistical support is provided for a wide range of NCI/NIH research. Some of this support involves using in-house programs to analyze mass spectrometry data to determine differential expression of proteins in tumors. This analysis identifies proteins and pathways that are affected and may be involved in tumor progression.

The HIV Dynamics and Replication Program is examining the variation in HIV sequences obtained from both DNA and RNA sequencing and is interested in the difference in sequences both within a given infected individual and between two individuals. The SAMM group is currently involved in examining sequences from infants to determine if they were initially infected by a single virus (known as the founder virus) or multiple viruses. By examining the differences between HIV sequences from a single infant, we hope to determine if the infection occurred at birth (the current assumption), or if they became infected while still in the womb.

The SAMM group also performed an analysis to determine whether socioeconomic factors may promote cigarette smoking. According to the CDC, cigarette smoking is the leading cause of cancer and death. Experimental studies provide evidence showing an overwhelming majority (90 percent) of lung cancers are caused by smoking. It is imperative to understand how social behaviors and disparities impact this preventable and highly addictive habit. Dr. Kelvin Choi (Division of Intermural Research, NIMHD) and SAMM scientists used the data from 2010–2011 Current Population Survey (n=170,469) to develop a predictive model that explains the intersectionality between the social features (education, retirement status, income, etc.) and smoking. SAMM scientists used a variety of statistical methods to develop a classification and regression tree (CART) model that was further used to identify key social features that seem to impact smoking behavior. Dr. Choi presented the work in the OBSSR 20th anniversary symposium.



CART model used to predict socioeconomic factors.

Educational Outreach

<u>NCI Outreach at the USA Science and Engineering (USAS&E) Festival</u>

The ABCC IVG presented computer visualization and virtual reality demonstrations at the NCI booth at the 2016 USAS&E festival. The demonstration attracted wide interest from large crowds of students and their parents. The following picture shows students and parents participating in the demonstration session.

The Imaging and Visualization Group's virtual reality demonstration session at the NCI Booth at the USA Science and Engineering Festival. "I really appreciate all you did to make the NCI Booth one of the most popular booths in the convention center." – Kathy McBrien, Project Manager, Office of Public Affairs, National Cancer Institute.

In addition, ABCC scientists from the SAMM group promoted good practices in data modeling by giving talks and organizing seminars for NCI and FNLCR staff and trainees.

Support Provided by the Clinical Monitoring Research Program

CMRP Program Management Office

The Clinical Monitoring Research Program (CMRP) Program Management Office provides program management and administrative support to the regulatory, clinical, and programmatic efforts for National Cancer Institute (NCI), National Institute of Allergy and Infectious Diseases (NIAID), Clinical Center, National Heart, Lung, and Blood Institute (NHLBI), National Institute for Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institute of Mental Health (NIMH), National Center for Advancing Translational Sciences (NCATS), and National Institute of Neurological Disorders and Stroke (NINDS) initiatives. This centralized office provides rapid responses and high-quality solutions to programmatic needs and recruits and retains diverse subject matter experts (SMEs) to successfully support the institutes' research missions.

The Program Management Office facilitates high-quality clinical research through program guidance and support, strategic planning and direction, technical direction, and general assistance to various government entities. Additionally, the Program Management Office mentors other non-CMRP programs within the Clinical Research Directorate (CRD) to achieve optimum practices to support the customers' expectations. Staff members provide detailed communications related to required training sessions and company policies and procedures, and provide direct support related to personnel requisitions and publication reviews.

The Program Management Office consists of an administrative director, a clinical program administrator, two senior program coordinators, an administrative coordinator, an administrative assistant, and a secretary.

The Program Management Office works closely with CMRP hiring managers and the Leidos Biomed Human Resources (HR) Directorate to recruit, interview, and hire qualified candidates for the numerous medical, clinical research management, regulatory, information technology, program/project management, and administrative positions within CMRP and in support of the many institutes. During FY2016, more than 60 positions were successfully filled, after completing approximately 300 phone, Skype/social media, and face-to-face interviews.

In response to a Yellow Task (YT) request addressing Zika, malaria, dengue, and other emerging viruses and infectious diseases, Program Management Office staff prepared nine new personnel requisitions to support the Clinical Trials Program within the NIAID Division of Clinical Research (DCR) Vaccine Research Center (VRC), and collaborated with the Leidos Biomed Scientific Publications, Graphics, & Media (SPGM) group to create a visually appealing advertisement to post in various active recruitment sites and social media channels. The flyer is expected to attract potential candidates to apply for open CMRP positions, which will support the conduct of clinical studies to develop, test, and advance vaccine candidates and approaches for building immunity protection.

The Program Management Office serves as a valuable resource for CMRP employees, providing orientation programs and logistical support to new hires while communicating and coordinating critical information pertaining to upcoming activities and staff responsibilities. The Program Management Office also prepares and submits credentialing packages to the NIH/NIAID Credentialing Office for professional positions supporting the institutes. Seventeen credentialing packages were submitted this period.

Policy and procedure updates are provided to all CMRP staff to ensure that the program operates under the most current guidelines and requirements. Under the direction of the CMRP director, the Program Management Office also works closely with the HR Compensation Department to coordinate the necessary performance management activities throughout the year. Due to recent telework policy changes, the Program Management Office evaluated CMRP needs and expected coverage during unscheduled closures, as well as confirmed existing approved telework agreements. The office distributed more than 230 Tier I/II/III memos and created/distributed for signature over 175 Unscheduled Closure Telework Agreements.

During the reporting period, the Program Management Office supported multiple international efforts, including those related to Ebola, respiratory viruses, and other infectious diseases. Staff developed more than 30 urgent Ebola personnel requisitions and facilitated approvals through the CMRP process for submission to HR for posting and active recruitment. Virtual private network (VPN) access account setup was also expedited for many of the international Ebola new staff hires. Staff worked closely with HR and the Division of Personnel Security and Access Control (DPSAC) to facilitate Ebola staff NIH Enterprise Directory (NED) accounts and obtain personal identity verification (PIV) badges to allow prompt access to all systems and computers.

The Program Management Office devised and implemented a communication plan for all off-site employees who support NCI/Center for Cancer Research (CCR) and the government customer. The plan effectively maps out processes, including recruitment/hiring, in-processing/out-processing, credentialing, leave notifications and requests, and performance evaluations. The plan designates a full-time point of contact for all Leidos Biomed NCI/CCR support staff and the customer and has proven to be effective in managing the relationships between staff members and the government customer.

During FY2016, the Program Management Office conducted an evaluation of the administrative support provided by patient care coordinators (PCCs) supporting the NCI/CCR medical support teams at NIH. While all PCCs have routine tasks to perform, some of the positions are utilized differently. Each PCC was shadowed for a half day in the clinic, and information was recorded on a spreadsheet to capture how PCCs support their teams and identify areas where PCCs could potentially provide additional support. The spreadsheet has become a useful tool when training PCCs on tasks, understanding various support activities, and showing how to incorporate additional tasks into routine functions. By utilizing PCCs more effectively, the clinical staff are able to focus more on direct patient care activities.

The Program Management Office organized quarterly training sessions for the PCC support staff located at NIH. The training sessions have proven to be a success, and staff members are pleased with the opportunity to enhance their professional development and grow within their current roles. The training sessions have not only been successful in complementing their knowledge base but have also provided a great opportunity for teambuilding and promoting a healthy work environment. Additionally, the office closely monitored required trainings for program-wide compliance, ensured all medical staff are adequately covered by malpractice insurance, and managed the CMRP process for reviewing publications (manuscripts, abstracts, external presentations, and posters) prior to submission to confirm compliance with prime contract requirements/guidelines.

CMRP Project Management Office

The CMRP Project Management Office provides project management and operational support to CMRP initiatives, as well as to several external high-profile projects such as the D.C. Partnership for HIV/AIDS Progress (DC-PFAP) between NIH and the U.S. Department of Health and Human Services (HHS). The Project Management Office comprises two clinical project managers, one medical writer, and one documentation specialist. Additional team members supporting the Project Management Office as well as other specific initiatives include one clinical project manager (NCI), one senior special projects administrator (NIAID), one medical writer, and two secretaries (NIAID).

Working in conjunction with the Program Management Office, the Financial Management Group (FMG), and other CMRP functional groups, the Project Management Office helps manage the YT system workflow for CMRP. YTs concerning high-priority initiatives are expedited to facilitate the efficient preparation of service, cost, and time estimates. The workflow process includes developing task responses, facilitating budget preparation and approval, creating and managing timelines, and tracking process metrics for CMRP. Under NCI's new Indefinite Delivery, Indefinite Quantity (IDIQ) task order contract vehicle, which began at the end of FY2015, Leidos Biomed implemented new operational and response processes and CMRP staff quickly defined methods to align with requirements and adapt to changes in the Leidos Biomed contract mechanisms.

Project Management Office staff coordinate the preparation and submission of annual and biannual reports throughout the contract year. These reports include the CMRP section of the Leidos Biomed Annual Report, the fall and spring Contract Performance Status Reports (CPSRs), monthly goals and objectives reporting, and the CMRP Report of Activities. The office continues to remain flexible, respond to inquiries, and adapt processes to support the collection of relevant and timely data to ensure comprehensive reporting of the program's work efforts.

The Project Management Office assists in establishing research subcontracts and agreements, including preparation of statements of work (SOWs), shepherding research agreement documents through the subcontracts process, and performing a critical review of proposals and budgets. The Project Management Office also supports the NCI Division of Cancer Control and Population Sciences (DCCPS) Behavioral Research Program Network on Biobehavioral Pathways in Cancer, managing several research subcontracts, including, but not limited to, the following projects: Medical College of Wisconsin, Pilot Study Using Propranolol to Decrease Gene Expression of Stress-Mediated Beta-Adrenergic Pathways in Hematopoietic Stem Cell Transplant Recipients; University of California, Los Angeles, fMRI Probe Optimization Study of Social Support; Monash University Phase 0 Study to Define the Effect of Perioperative β-blockade on Biomarkers of Progression in Newly Diagnosed Breast Cancer; Rutgers Robert Wood Johnson Medical School, Stress and Tumorigenesis in Normal and p53 Knockout Models; Massachusetts General Hospital, Pilot Study of Oncologists' Affective and Decisional Processes for Administering Chemotherapy at the End of Life; Columbia University Medical Center, Proof of Concept: N of 1 Trial Design for Behavioral and Psychological Issues in Cancer Survivorship; and Washington University in St. Louis, Systematic Reviews to Inform Research and Treatment for Multi-Morbidities.

Furthermore, the Project Management Office manages the subcontracting efforts for the CCR's protocol re-engineering strategic planning projects with Dilts + Partners, LLC, and NIAID's Development of Program Performance Measures in DCR. The Project Management Office also oversees NIAID DCR research subcontracts that are not associated with any of the larger DCR initiatives yet require project management support. Many of these initiatives fall under the NIAID Clinical Consulting and Support (CCS) section of this report.

The Project Management Office provides background research for projects, assists with preparing presentations, edits SOWs, reviews job requisitions, generates meeting minutes, writes internal procedures, and assists the Regulatory Compliance and Human Subjects Protection Program (RCHSPP) Clinical Safety Office (CSO) with protocol reviews.

The Project Management Office also takes the lead in supporting CMRP technical project managers (TPMs) responsible for comprehensive support to research subcontracting and other acquisitions activities. Support includes the dissemination of subcontracting policies, guidelines, and processes, defining workflow processes, establishing procedures and training, ensuring consistent communication across CMRP, and serving as a resource to mentor new TPMs.

CMRP Learning and Professional Development

It is the mission of CMRP's Learning and Professional Development (L&PD) group to provide quality continuing education and training programs that address regulatory, technical, and professional skills competency, and encourage professional development by identifying and facilitating training events for our diverse internal and external customers.

The L&PD group is composed of a clinical training manager, two training specialists, and a training coordinator. The L&PD group supports CMRP staff, the NIAID DCR Office of Clinical Research Policy and Regulatory Operations (OCRPRO), and the NIAID Office of Planning and Operations Support (OPOS).

L&PD activities fall into four categories: providing training sessions to address client-identified needs, providing training and development subject matter expertise, providing administrative support for activities with training implications, and ensuring compliance and continuous improvement of training processes and initiatives. Detailed descriptions of these activities are provided below.

L&PD provided internally developed and focused training sessions to address customer-identified needs, as well as a myriad of training and professional development opportunities for CMRP's geographically diverse staff. The training sessions included workshops on the Myers-Briggs Type Indicator (MBTI) and customized sessions on the impact of MBTI on critical professional skills, e.g., communication, becoming a facilitator for emotional intelligence training, providing assertive communication techniques to assist a team in coping with aggressive communication, and developing and delivering training on time management, as well as creative problem solving and decision making for a team for whom these skills are critical to success with its customers.

CMRP L&PD continues to host numerous training opportunities, including 22 New Employee Orientation (NEO) sessions, three Monthly Seminar Series, and the Deputy Director of Management (DDM) series. L&PD also hosted the Ethical and Regulatory Aspects of Clinical Research course for seven staff members.

The clinical training manager continued to provide several sessions to the Frederick National Laboratory for Cancer Research (FNLCR) in collaboration with HR. These sessions included Managing Up, Influencing without Authority, Influencing without Using Your Authority, and Generations in the Workplace.

L&PD continues to maintain hard copy and electronic versions of the training records for approximately 250 employees. Additionally, L&PD administered the 2016 résumé and curriculum vitae, signature log, and license/certification review for all CMRP staff to ensure 100 percent compliance with the Good Clinical Practice (GCP)-regulated environment.

Training and professional development are critical activities for numerous clinical research and medical personnel. Continuing education units (CEUs) are a requirement for maintaining licenses and certifications. Fiscal constraints and the HHS Efficient Spending Policy have significantly restricted the staff's ability to attend relevant conferences and training events. During the reporting period, L&PD continued to offer International Association of Continuing Education and Training (IACET) CEUs for instructor-led training sessions provided by CMRP SMEs and also adapted its processes to provide IACET CEUs for instructor-led training sessions provided by third-party vendors. This provides a significant cost savings for those who need to accumulate CEUs to maintain professional certification.

CMRP Information Technology

The CMRP Information Technology (IT) group provides software development and computer, network, application, and disaster recovery support to NCI, NIAID, Clinical Center, NHLBI, NIAMS, NCATS, NIMH, and NINDS initiatives. Members of the IT group specialize in evaluating core business processes and using simple, flexible methodologies to transform business needs into suitable, cost-effective technical solutions while satisfying customer requirements and meeting the unique operational requirements for managing clinical trial, regulatory, and clinical safety data. The IT group continually assesses the goals and objectives of CMRP and uses leading-edge technology to provide the best return on investment, while ensuring compliance with all applicable regulatory and security best practices, policies, and standards.

During the past year, the IT group was involved in several key technical initiatives for the program, as described below:

IT Infrastructure Support

The IT group continues to maintain and operate a CMRP IT infrastructure within the Building 430 data center to better support CMRP operations and groups located throughout the NIH community. Maintaining the technical framework is a collaborative effort of staff from the Data Science and Information Technology Program and the CMRP IT groups. The operational environment provides program and technical staff members with the capability to use multiple platforms for testing and developing new software or system functionality enhancements; to store program materials, financial reports, and similar content; and to electronically manage business processes. Integrated disaster recovery services

and capabilities are supported within the technical framework, with the release of a fully redundant, automated tape backup library system that uses fiber optic channel cards to collect data from the servers.

During FY2016, the IT staff member appointed as the Health Insurance Portability and Accountability Act (HIPAA)/Health Information Technology for Economic and Clinical Health (HITECH) Act compliance officer continued to review and provide guidance on incident reports submitted by program staff, ensured appropriate safeguards were in place and necessary actions were taken, and provided follow-up when indicated. Additionally, the CMRP IT group provided continued support of the smart card authentication requirements and standards set forth by Homeland Security Presidential Directive 12 (HSPD-12) and associated Federal Information Security Management Act regulations, Office of Management and Budget memoranda, and NIH policies.

The IT group participated in the annual FNLCR intrusion detection tests to assess network host vulnerabilities and in post-remediation efforts to mitigate findings. This exercise was essential in providing a comprehensive review of potential threats to the program's IT assets based on the FNLCR campus and in identifying changes that need to be made to existing business processes to prevent future threats.

CMRP IT staff continues to support the digital signature initiative introduced in FY2012, in which HHS-verified Public Key Infrastructure software certificates embedded on individual PIV cards were utilized to digitally sign Adobe Acrobat program documents in lieu of paper-based, wet signatures for approvals. This effort has been very beneficial to the program, as it has reduced the time it takes to obtain approvals. This initiative continues to have a cost benefit and a positive environmental impact.

Websites

The CMRP IT group provides maintenance and technical support for a public-facing website that highlights the diverse clinical research support services provided by CMRP. The site provides information about the many high-profile NCI, NIAID, Clinical Center, NHLBI, NIAMS, NCATS, NIMH, and NINDS initiatives to investigators, clinicians, prospective job seekers, and the general public. Content management support, bug fixes, and similar activities continue to be provided for this site.

Mobile Device Refresh Program

To best serve the growing mobility needs of our internal program clients, the mobile device refresh program continues to be supported by the IT group to evaluate equipment being used within the program, establish prioritized replacement schedules, acquire and configure the new equipment, complete applicable property accountability and loan documents, and facilitate the return and final disposition of existing devices.

Support for West Africa Ebola Clinical Research Initiatives

Several initiatives were undertaken to provide IT support for the Ebola clinical research activities at many different locations in West Africa. The support efforts to these initiatives are included in the NIAID Division of Clinical Research (DCR) section of this report.

CMRP Financial Management

The CMRP FMG provides comprehensive support to CMRP staff and continues to expand its capabilities in support of NCI, NIAID, VRC, NHLBI, NIAMS, NIMH, NINDS, NCATS, and the Clinical Center.

FMG collaborates with other Leidos Biomed directorates, programs, and departments, as well as with government officials, and manages 135 project IDs, mostly in support of NCI and NIAID.

- Office of the Directorate – 2
- NCI Immediate Office of the Director (OD) – 21
- NCI Division of Cancer Epidemiology and Genetics (DCEG) – 4
- NCI CCR – 50
- NCI Division of Cancer Treatment and Diagnosis (DCTD) – 14
- NCI DCCPS – 1
- NCI Division of Cancer Prevention (DCP) – 1
- Clinical Center – 3
- NIAID Division of Intramural Research (DIR) – 10
- NIAID DCR – 20
- NIAID Division of Acquired Immunodeficiency Syndrome (DAIDS) – 3
- NIAID VRC – 1
- Other institutes – 5

In addition, the CMRP FMG provided oversight of 17 CMRP/NIAID/Vaccine Clinical Materials Program (VCMP) project IDs, which CMRP manages in collaboration with the Vaccine Pilot Plant (VPP).

During FY2016, FMG completed the FY2017 annual budget preparation process for submission-based contract deadlines. FMG expanded the capabilities of its SharePoint site in support of NIAID DCR and NCI to include real-time tracking of actual expenses against planned assumptions, enabling FMG to maintain estimates at completion (EAC), track detailed information related to the efficient spending policy, and share information with management, NIAID DCR, and NCI government counterparts in an expeditious manner. This improved the program's operational efficiency.

FMG also continued to manage and develop cost estimates for new and revised work scopes, provide monthly static financial report information, and track project expenses for all budgets to ensure the accuracy and accountability of all costs.

With the awarding of new IDIQ task orders, the CMRP FMG worked closely with senior management and TPMs to forecast EACs on existing non-severable efforts and to manage the transition of staff from the existing projects in order to continue support on newly approved IDIQs.

CMRP FMG worked closely with CMRP senior managers and the Leidos Biomed Business Office to coordinate the addition of new project IDs in support to NCI CCR, IOD-Center for Global Health (CGH), IOD-Coordinating Center for Clinical Trials (CCCT), CMRP/VCMP, VRC, DCCPS, NIAID-DIR, and NIAID-DCR in order to satisfy the requirements of the new Enterprise Resource Planning (ERP) system. In addition, the CMRP FMG was instrumental in coordinating specialized information sessions with new staff that joined the program during the past year. Sessions included training on the ERP Cognos system to expand skills and knowledge for running reports in preparation of EACs, and monthly tracking of expenses and encumbrances by project.

CMRP FMG worked closely with TPMs to monitor funding balances to ensure that the total expenses, commitments, and encumbrances for all non-severable activities meet the deadlines for funding that will expire in FY2016. This also required working closely with senior management, Leidos Biomed auditors, the Leidos Biomed Financial Planning and Analysis Office, and subcontracts to track, analyze, and continuously update reports in an effort to maximize funding utilization.

The CMRP FMG worked closely with the VCMP, Leidos Biomed Finance, and a CMRP senior TPM and project manager to transfer the financial oversight responsibility of a portion of the VCMP's BioD Clinical Material Manufacturing projects in support of vaccines for chikungunya, malaria, Western, Eastern, and Venezuelan Equine Encephalitis (WeVee), Zika, and Ebola for the overall project management of 11 new project IDs for site feasibility, site initiation, monitoring, and close-out of sites in support of the VCMP. Collaboration with this new Leidos Biomed customer benefitted from similar support provided to programs supported by CMRP. In addition, the CMRP FMG worked closely with the CMRP technical representative to develop a multi-tabbed financial tracking file that, after each project was updated, automatically updated three other reports based on different customer needs.

Travel Coordination

A program manager coordinates all aspects of the HHS Efficient Spending Policy for CMRP. The policy requires new and additional approvals for non HHS-sponsored conference attendance and NIH-sponsored conferences and meetings. The CMRP program manager communicates all policy updates, requirements, and deadlines to CMRP staff as they become available from the respective HHS/NIH divisions and agencies to ensure that all data call deadlines are met. During this reporting period, CMRP went from quarterly data calls to rolling data calls to monthly data calls. CMRP staff have continued to demonstrate flexibility and accountability by meeting the required reporting dates.

A high volume of travel to West Africa continued in FY2016 to support Ebola clinical research efforts; CMRP's efforts are discussed further in the NIAID DCR section. The CMRP program manager received an NIH Merit Award in November 2015 for exceptional team performance in coordinating nearly 340 complex travel arrangements to three Ebola-stricken countries and receiving outstanding traveler feedback.

As requested by the NCI at Frederick Office of Scientific Operations in August 2015, CMRP has been making progress on the corrective action to reduce the number of emergency travels (less than four weeks from departure date) submitted. CMRP turned this effort into a formal Goal and Objective (G&O) and successfully reduced the number of emergency travel requests; however, this is still a difficult task for a number of travel requests due to the different entities and players involved. CMRP continues to monitor the emergency travel requests, assess improvement opportunities, and make updates to the CMRP Travel Standard Process document.

After the Brussels Airport bombing attack in March 2016, CMRP implemented an international traveler emergency preparedness process to ensure smooth communication and rapid, consistent support for CMRP staff in the event of a serious emergency abroad. Now, CMRP staff traveling internationally are provided with pertinent information they may need immediate access to in the event of an emergency and are encouraged to enroll in the U.S. Department of State (DOS) Smart Traveler Enrollment Program (STEP).

In response to evolving threats in various countries, the U.S. DOS increased the security training required for U.S. government personnel who travel to certain foreign locations. Although this training is not required for Leidos Biomed travelers, CMRP staff traveling to designated countries must complete the High Threat Security Overseas Seminar (HTSOS) online course.

CMRP Document Control

The CMRP documentation specialist coordinates, compiles, and tracks all program documents and maintains the CMRP master documentation file and archive. The document specialist creates progress report templates to assist TPMs with managing subcontractor progress reporting. Additionally, the documentation specialist provides administrative and clerical support related to program initiatives.

A web-based document management software system is used to collect, store, and summarize data on publications by CMRP employees, ensuring that program reports are in compliance with NIH requirements. The documentation specialist manages and coordinates the CMRP internal publication review process and works with staff members and customer principal investigators (PIs) to ensure the correct affiliation and funding statements are listed on each publication.

The documentation specialist continues to draft and update sections of the CMRP Report Handbook to define staff roles and responsibilities in writing, reviewing, and submitting program reports.

During FY2016, the documentation specialist also assisted in the maintenance of a CMRP research subcontracts database, helped track and load financial updates into the SharePoint library, maintained a subcontract spreadsheet for research agreements, managed a spreadsheet to track historical YT data, and assisted with subcontract renewals.

The documentation specialist assisted in the management of all contractual reporting requirements by creating report section templates, monitoring and updating the report tracker, and creating a project ID mapping tool to align all IDs to their specific report section, government customer, and TPM.

The documentation specialist also created an Excel invoicing template for several program initiatives and assisted with the organization and logistics of the DCCPS Network Capstone Meeting at NCI.

Technical Project Management Support Provided by the Applied and Developmental Research Directorate

Biorepository Subcontract, Fisher BioServices, Inc.

The mission of the NCI at Frederick Central Repository is to support the research programs of the NCI, National Institutes of Health (NIH), /NIAID/NIDDK clients and other groups. The primary core functions of the Central Repository are the receipt and distribution of biomaterials for research and clinical testing, and temperature appropriate storage of over 15.5 million biological specimens. The Central Repository has the capability and capacity to store biological materials at temperatures which include: ambient, 4ºC, -20ºC, -40ºC, -80ºC, -150ºC and -196ºC.

The Central Repository stores these biological materials in strict accordance with the International Society for Biological and Environmental Repository (ISBER) Best Practices and the NCI Best Practices for Biospecimen Resources. The Central Repository's dedicated staff is experienced in the domestic and international shipments of biospecimens in compliance with current International Air Transport Association (IATA) and Department of Transportation (DOT) standards.

Infrastructure: The robust designs of all three repository locations are capable of accommodating all types of commercially available biospecimen storage mechanical freezers and cryogenic units. The NCI at Frederick Central Repository facilities consist of standard administrative offices, BL-2 compliant sample processing laboratories, and secure biospecimen storage facilities. All repository facilities have dedicated LN_2, electrical and HVAC systems with redundant backup multi-fuel electric generators, and multiple building security features/ systems; including an integrated fire alarm system with multiple methods of fire suppression and oxygen level monitoring. In addition, there is complete 24/7/365 alarm monitoring of all critical building support systems, facility spaces, and sample storage units. All repositories are controlled entry facilities that can only be accessed by authorized personnel having an issued key card.

Weather Closings: The Frederick National Laboratory for Cancer Research (FNLCR) experienced numerous base closings this winter (2014–2015) due to inclement weather. Fisher Bioservices, Inc., staff continuously monitored repository sample storage equipment and facilities in the event of an emergency or equipment failure for the duration of these weather related events.

Initiatives: Leidos Biomedical Research Inc. (Leidos Biomed) was successful in the recompete of the NCI at Frederick Central Repository subcontract. The new subcontractor, American Type Culture Collection (ATCC), will be completely transitioned into this role by the end of the contract year. ATCC is a global leader in research and development expertise and will provide the NCI at Frederick Central Repository with cutting edge technology.

Technical Project Management Support Provided by the Management Support Directorate

Scientific Library Subcontract, Wilson Information Services Corporation

Through a subcontract with Wilson Information Services Corporation (WISCO), QMO provided technical oversight of operations at the NCI at Frederick scientific library. As part of this effort, the QMO worked with WISCO to evaluate software platforms to collect and report altmetrics for NCI-F publications.

Coordinating Center for Clinical Trials

Support Provided by the Clinical Monitoring Research Program

The Coordinating Center for Clinical Trials (CCCT) was established in 2006. Its overarching goal is to strengthen NCI's clinical trials and translational research enterprises, as well as promote cooperative endeavors that draw upon the strongest components of the clinical research system and scientific infrastructure, and include constant engagement of critical stakeholders.

CCCT guides the implementation of recommendations made by the National Cancer Advisory Board (NCAB), Clinical Trials Working Group (CTWG), and Translational Research Working Group (TRWG). This implementation is achieved through continuous dialogue with NCI leadership and scientific staff, clinicians, researchers, advocates, policymakers, industry, and foundations, in order to enhance the effectiveness of the

nation's cancer clinical research enterprise. CCCT oversees implementation of the 22 initiatives recommended by the CTWG in 2005 as well as 15 initiatives recommended by the TRWG in 2007.

In 2008, CCCT made its first request for CMRP to support the NCI Scientific Steering Committees (SSCs). Since then, three additional YTs have been approved to support CCCT. During the reporting period, staff supported three distinct activities: (1) 17 NCI Steering Committees (SCs); (2) the Biomarker, Imaging, and Quality-of-Life Studies Funding Program (BIQSFP); and (3) CCCT Meeting Planning/Travel (MP/T).

The clinical project managers maintained consistent and effective communication between CMRP and CCCT program directors and continued to be an integral part of the CCCT team. Currently, the CMRP CCCT support team comprises seven full-time equivalents (FTEs), with the potential to increase to 10 FTEs if necessary.

NCI Steering Committees

Between 2008 and 2016, the SC program increased from six SCs and 140 consulting agreements to the current 17 SCs and 511 vendor agreements. In support of the SCs, CMRP staff provided project management support, program analysis, and management of the large vendor agreement effort, which includes quarterly invoicing and payments to vendors based on SC attendance.

CMRP continued to maintain the CTWG/CCCT research database, which contains conflict-of-interest and confidentiality disclosure agreement documents, as well as term dates for the 511 SC and task force members. Staff also supported weekly CCCT meetings, provided progress reports on assigned projects, and designed slide presentations for CCCT program directors.

The CMRP CCCT support team was also tasked with coordinating the election of community oncologists to the SC/task force. This effort was based on a recommendation from the CTWG report requiring community oncologist involvement in clinical trial design and polarization through representation on the NCI SC and task force.

The CMRP CCCT support team also supported, coordinated, and hosted webinars within the CCCT for NCI task forces and working groups. The clinical project manager continues to be the go-to person for CCCT-sponsored webinars. In addition, CMRP continued to provide updates, maintain the CCCT websites, and support the Clinical Trials Planning Meeting (CTPM) and SC in-person meetings. The clinical project manager also coordinated the effort to create a shared folder on the CCCT network drive where all stakeholders can easily share and access SC-related information.

The clinical project manager served as a member of the Patient Advocate Steering Committee (PASC) Agenda Planning team tasked with planning the April 15–16, 2016 in-person meeting.

The clinical project manager, working with another contractor, EMMES, designed fillable versions of the Confidentiality Disclosure Agreement (CDA) and Conflict of Interest (COI) forms, streamlining the process for completing these forms. The forms were revised in May 2016 to allow for database entry of information. This procedural modification has resulted in cost savings, has simplified CDA/COI acquisition and processing, and has allowed for e-archiving of the signed forms.

Biomarker, Imaging, and Quality-of-Life Studies Funding Program

CMRP continued to support the NCI BIQSFP, facilitating the robust NCI SC evaluations of more than 22 BIQSFP applications. Twelve BIQSFP studies have been approved by the Clinical and Translational Research Operations Committee (CTROC) for funding during the period of performance, with an additional four potential studies for approval prior to the end of the period. CMRP staff also continued to support 15 active research subcontracts in support of the NCI National Clinical Trials Network (NCTN).

CMRP staff facilitated and supported the December 2015 revision of the BIQSFP announcement, including updating the announcement on the official BIQSFP website. The main CCCT website, developed by CMRP several years ago, has consistently received 2,500 to 3,000 page views per month, including the BIQSFP pages, from U.S. and international visitors to the site.

The BIQSFP Huddle Log, developed by CMRP and instituted in FY2014, continued with weekly meetings to ensure the monitoring and progress of BIQSFP studies under evaluation.

CMRP staff also centralized data on all BIQSFP studies into one Excel workbook, which includes the ability to search, sort, and filter data, and provides a central repository for financial files.

Meeting Planning/Travel

The Meeting Planning/Travel (MP/T) team began supporting CCCT in April 2013. During FY2016, CMRP staff successfully planned and facilitated 16 meetings and one webinar for CCCT, the Federal Advisory Committee Act (FACA), DCTD Specialized Programs of Research Excellence (SPORE), and the DCTD Immunotherapy Working Group. MP/T planning activities are underway for nine additional meetings through the end of FY2016.

MP/T staff provided on-site meeting support in Chicago for 45–50 NCI CCCT/Cancer Therapy Evaluation Program (CTEP)/DCTD meetings during the 2016 American Society of Clinical Oncology (ASCO) Annual Meeting. These meetings took place over a four-day period, and 15–18 meetings were held each day.

The MP/T team's role in the CCCT Integrated Meeting Planning Team continued to grow due to additional NCI CCCT administrative staff changes. CCCT now relies on the MP/T team to prepare all components of the HHS Conference Approval Form (CAF) packages and the NIH 827-1 Form.

Center for Global Health

Support Provided by the Clinical Monitoring Research Program

The Center for Global Health (CGH) supports NCI's goal to advance global cancer research, build expertise, exchange scientific knowledge, and leverage resources across nations to address the challenges of cancer and reduce cancer deaths worldwide. CGH facilitates research efforts by collaborating with other NCI divisions, U.S. and foreign government agencies, non-government organizations, and pharmaceutical and biotechnology companies, and by supporting cancer research networks in low- and middle-income countries (LMICs) to increase global capacity for cancer control and research.

Meeting and Conference Support

CGH is NCI's principal vehicle for coordinating and prioritizing its global cancer research activities. Specific objectives are to (1) create sustainable international partnerships, (2) support programs that address global gaps in research and scientific training, and (3) disseminate information and best practices that drive improvements in cancer research and control. Through a sustainable, international, and integrated network of partners, CGH collaborations serve as a catalyst for reducing the global cancer burden by using NCI's professional expertise to facilitate worldwide health diplomacy; develop and enhance research capacity; assess cancer burden; develop and validate new agents and diagnostics for cancer prevention, detection, and treatment; share knowledge; identify new research opportunities; and support programs that address global gaps in research and scientific training.

To conduct these activities, CGH hosts conferences, investigator meetings, protocol meetings, training sessions, and workshops both domestically and internationally. This effort is comprehensive and requires support from many NIH institutes and other collaborators.

Scientific and clinical research programs, training programs, and technology and capacity-building programs are among the integrative initiatives and programs that are assisting CGH in achieving its goals.

The Scientific and Clinical Research Program allows investigators to conduct high-quality clinical cancer research that provides important information on treatment response and related pharmacogenomic pathways. The Training Program ensures that the cancer research networks are sustainable by developing specific training opportunities to cultivate a robust pipeline of basic and clinical investigators. The Technology and Capacity-Building Program provides an overarching framework for conducting cancer research, adapting advanced technologies and methodologies, and managing biospecimens, informatics, and data management, with the intention of advancing research capacity and developing sustainable research infrastructure.

CMRP staff support the goals of CGH by planning and coordinating meeting and travel activities related to domestic and international meetings, conferences, and training for both government and non-government attendees who are collaborating on many initiatives and programs. Services include: (1) providing comprehensive logistics support; (2) preparing and monitoring meeting and travel budgets; and (3) establishing formal processes and procedures to streamline planning, while ensuring that all meeting and travel policies and directives are followed.

CMRP staff was instrumental in the planning and coordination of 13 major meetings. Domestic meetings included: Global Health Week in Rockville and Bethesda, MD; the Symposium on Global Cancer Research (CGH/CUGH) in San Francisco, CA; and the Pink Ribbon Red Ribbon Steering Committee meetings in Bethesda and Rockville, MD. International meetings included: the Central Asia Leadership Forum in Tashkent, Uzbekistan; the International Society of Pediatric Oncology (SIOP) Paediatric Oncology in Developing Countries (PODC) Cancer Control Workshop in Cape Town, South Africa; the WE CAN Breast and Cervical Cancer Advocacy, Education and Outreach Summit held in Bucharest, Romania; the Kenya Cancer Research Forum in Nairobi, Kenya; the International Conference on Betel Quid and Areca Nut in Kuala Lumpur, Malaysia; the Asia-Pacific Cancer Control Leadership Forum in Singapore; the Kenya Stakeholder Forum in Nairobi, Kenya; Minsk Engagement in Minsk, Belarus; the Latin American Leadership Forum in Antigua, Guatemala; and the APEC Cervical Cancer Prevention and Control Workshop in Lima, Peru.

The CGH deputy director requested that the CMRP team develop the budgets for all of its meetings, recognizing that the team is more knowledgeable than CGH staff given the volume of travel activities the team coordinates. The CMRP team can provide a budget in one to two hours, whereas CGH staff may require up to one week. Given the success of this team, it was tasked with supporting an urgent meeting for the Ebola project.

As a result of working at a global capacity, two of the biggest challenges are the time differences between the United States and the meeting locations and the language barriers. The team addresses these challenges by maintaining enhanced communication and collaboration with CGH, who co-sponsors the meetings, and with the travelers. This heightened attention to effective communication ensures that all stakeholders are kept well-informed of the meeting logistics/details and meeting/travel policies and guidelines.

The CMRP team continues to tackle the challenge of educating the CGH customer about travel requirements. The senior program coordinator was invited to one of CGH's all-hands staff meetings to present about travel policies for supported travelers. The presentation was well-received, and the senior program coordinator has asked to be invited back to present on a quarterly basis.

Research Subcontract Support: Subject Matter Experts

Given the outstanding support provided by the CMRP team to the CGH meeting, planning, and travel activities in July 2015, CGH requested additional support from Leidos Biomed to establish consulting agreements with subject matter experts to support the Cancer Research in the Media Workshops.

During the reporting period, CMRP staff managed nine consulting agreements to ensure sufficient labor and travel funds were available to complete the ongoing/ expanded work; careful attention was required to identify the appropriate timing for subcontract modifications required to increase the subcontract value. Two subcontracts were already in place for curriculum development and workshop presentations. The CMRP clinical project manager completed comprehensive pre-award and initiation activities for an additional seven agreements to collaborate and coordinate with CGH principals: (1) in support of the Planning, Partnership and Outreach team within the Planning, Evaluation and Operations (PEO) Branch; (2) to develop collaborative relationships and nurture partnerships among ministries of health, academic institutions, and non-government organizations for the further development and implementation of the palliative care activities in Central Asia; (3) for the further development and implementation of the evidence-based cancer control planning and cervical cancer activities in Asia; (4) to provide overall advice and guidance to senior staff, serving as a special advisor for CGH; (5) to provide training to CGH staff and partners at domestic and foreign locations, serving as the lead presentation and media coach for CGH; (6) to provide in-country technical assistance in surveillance and cancer control planning to the Kenya Ministry of Health and partners; and (7) to conduct the formal evaluation of the cervical cancer control program in Kenya. Of the nine agreements, three will be completed by the end of FY2016 and six will be renewed to continue into FY2017. An additional four agreements will be initiated in early FY2017.

One of the challenges encountered by Leidos Biomed staff is the short deadline to execute agreements and modifications due to underestimating the time and travel requirements. Multiple urgent modifications have been required to address additional work/time necessary to meet the CGH expectations and the increased funds to cover the additional labor and travel. CMRP staff continue to collaborate with the CGH deputy director to encourage all CGH staff to be more thoughtful about comprehensive full-cycle agreements and to make full annual funding available in order to avoid multiple agreement modifications.

Pilot Collaborations with LMICs in Global Cancer Research or Global Health Research at NCI-Designated Cancer Centers

Cancer is a leading cause of death worldwide, with a disproportionate burden occurring in LMICs. It is estimated that by 2020, new cancer cases will reach more than 16 million, and the majority of this burden will be borne by residents in LMICs. In 2013, CGH and the Office of Cancer Centers (OCC) developed a funding opportunity to promote research collaborations between NCI-Designated Cancer Centers and institutions in LMICs to stimulate cancer research pilot programs and expand the reach of cancer centers in international settings. The scope of these pilot proposals was broad and included a range of research projects, trainings, advanced technologies, clinical research network development, and other focus areas to support the development of cancer research capacity in LMICs. Evidence from this research will help populations not only in LMICs, but also in the U.S.

To support the LMIC pilot project and the work performed by the centers and their collaborating foreign partners, research subcontracts managed by Leidos Biomed were awarded in early 2014. The purpose of this effort was to stimulate cancer research pilot programs and expand the reach of cancer centers in international settings. This initiative was a unique opportunity to strategically develop partnerships between regional institutions and U.S. cancer centers, which leverage the scientific expertise and leadership in cancer control, prevention, and treatment to address new research directions as opportunities.

The scope of the initiative included a range of clinical research projects, training opportunities, advanced technologies, and clinical research network development. These and other focus areas supported the development of cancer research capacity in selected countries in Africa (Ghana, Nigeria, Tanzania, Malawi, Zambia, Uganda, and Kenya), Central America (El Salvador and Honduras), South America (Chile and Brazil), and Asia (two projects in India). The projects have helped CGH achieve its goals of fostering scientific and clinical research collaborations, expanding opportunities for training to cultivate a robust pipeline of basic and clinical investigators, and expanding technology and capacity-building programs. Investigators have been able to conduct high-quality research by adapting advanced technologies and methodologies for informatics, data management, and biospecimen management; developing sustainable research infrastructures; providing important information on treatment response; and ensuring that developing cancer research networks are sustainable.

Project work was completed by late 2015, and it is anticipated that the findings and discoveries from this collaboration will support continued improvements in cancer control, prevention, and treatment strategies both in the U.S. and abroad. During FY2016, CMRP continued to provide programmatic and subcontract management and internal audit support to the LMIC project in order to close out the 15 research subcontracts.

Global Cancer Project Map

CMRP provided administrative, technical, and strategic support to the Global Cancer Project Map (GCPM), which launched in March 2015 as a partnership

between the NCI CGH and Global Oncology, Inc. (GO), a non-profit organization. The Map, a first-of-its-kind online tool, showed international efforts related to cancer research, care, and outreach by integrating cancer control program and research project information from various organizations worldwide.

The Map's interactive features allow users to: (1) search for collaborators and projects by cancer type, project type, country, organization, and funding source; (2) visualize information pertinent to each project on an interactive world map; (3) initiate contact with PIs and program directors; and (4) overlay country-level epidemiological measures that provide a representation of the burden of cancer. GCPM's primary goals with the project were to: (1) establish and maintain a web-based central repository of internationally focused cancer projects; (2) facilitate the building of collaborations across organizations; and (3) accelerate progress, ensure a balanced investment of resources, and align global cancer research and control efforts.

The CMRP GCPM manager provided overall project management support to oversee the day-to-day work of the research subcontractor, GO, which was the primary mechanism for work done on this project. In addition, the CMRP TPM provided overall subcontract management, including the technical review of project deliverables, monitored the project's operating budget, and supported meetings between the Leidos Biomed, NCI CGH, and GO project teams. The research subcontract with GO supported a sub-agreement between GO and a web developer to build and maintain the GCPM website, and programmatic work related to outreach to organizations for project data, data cleaning, and data uploads to the GCPM, and promotional activities.

The CMRP GCPM manager provided administrative, technical, and strategic support to GCPM, working with NCI CGH to carry out the Center's involvement in the Map and ensuring the completion of project/subcontract milestones. The CMRP GCPM manager conducted weekly meetings with the CGH, Leidos Biomed, and GO project teams to address data questions, uploads, and technical development activities, as well as biweekly meetings with the full CGH/Leidos Biomed/GO project teams to provide updates and to plan for project work in four main substreams: administration, data, promotions/ publications, and partnerships. Additional support included leading data cleaning efforts, reviewing data sets posted to the Map, leading promotional opportunities for CGH, and facilitating the development and review of publications. The CMRP GCPM manager presented Map information on behalf of CGH at annual meetings of the Association of American Geographers (AAG), International Cancer Research Partnership (ICRP), and the 2016 Geographical Information System (GIS) and Health Symposium, sponsored by the American Public Health Association (APHA) and Urban and Regional Information Systems Association (URISA).

During FY2016, several significant accomplishments occurred: new data was added to the Map, data quality was improved across all Map projects, and a GCPM Partnership Model was initiated. At the end of the subcontract with GO (August 5, 2016), GCPM contained 1,800 projects (up from 1,400 in October 2015). The projects displayed on the Map are from NCI grantees, five NCI-designated cancer centers, the Union for International Cancer Control (UICC), the American Society of Clinical Oncology (ASCO), and the African Organization for Research and Training in Cancer (AORTIC).

The GCPM project teams actively promoted the Map at the APHA Annual Meeting in November 2015 (Chicago); the AORTIC Biannual Meeting in November 2015 (Morocco); the AAG Annual Meeting in March 2016 (San Francisco); the 4th Annual Global Cancer Symposium in April 2016 (San Francisco); the Consortium of Universities for Global Health (CUGH) Annual Conference in April 2016 (San Francisco); the International Cancer Research Partnership (ICRP) Annual Meeting in April 2016 (Atlanta); the International Agency for Research on Cancer (IARC) 50th Anniversary Conference in June 2016 (Lyon, France); and the URISA and APHA GIS and Public Health Symposium in June 2016 (Washington, D.C.). Twitter messages were prepared for the NCI CGH and GO Twitter accounts around every conference, and website user statistics have been analyzed using Google Analytics.

The GCPM project faced challenges with timely and accurate uploads of data via the GCPM website, largely due to issues with the website data upload feature. The CMRP GCPM manager and the CGH and GO project teams used the delay periods as an opportunity to improve the data quality of projects currently on the Map by creating and applying data-cleaning standards, identifying project duplications on the Map, pulling all projects into one centralized master file, and adding new project data to the master file. These data-cleaning efforts, largely led by the CMRP GCPM manager, resulted in much-improved utility and navigation of the data on the GCPM.

With the support of the CMRP GCPM manager, CGH obtained a higher level of attention to the GCPM project. Having a full-time dedicated staff person allowed CGH to identify areas of improvement for GCPM, increased documentation of project status updates and project challenges, and increased CGH's ability to carefully manage the GCPM reporting milestones. This level of support to the GCPM project increased engagement, particularly from CGH leadership. Ultimately, CGH and CMRP worked together to determine that the technical and partnership challenges that existed between the CGH vision for GCPM and the research subcontract vendor's vision for GCPM created fundamentally different long-term visions for the Map. In July 2016, it was determined that it was in the best interest of CGH and its resources to end work with the vendor at the end of the subcontract period of performance.

Despite the decision to sever the collaboration with GO, GCPM was relevant to NCI CGH because it is a useful tool for understanding what types of research and programmatic work are being done in a particular country and identifying potential expansion opportunities. CGH leadership frequently travels to international cancer meetings, works to organize international research programs, and provides technical assistance for global cancer control.

Even with the conclusion of GCPM work, CGH remains very interested in geospatially mapping cancer research and control projects around the world and plans to translate the experience gained from the GCPM project into a new cancer research mapping project with a different partner. Going forward, the CMRP GCPM manager will continue to support CGH in the development of a new global cancer research map for the ICRP, an organization that CMRP and CGH staff spent considerable time with this past year developing significant buy-in and interest for the addition of a geospatial mapping platform to the ICRP website.

CGH Evaluation Services

To support CGH's mission of creating sustainable international partnerships, supporting programs that address global gaps in research and scientific training, and disseminating best practices that drive improvements in cancer research and control, NCI requested that Leidos Biomed provide program evaluation support services. CMRP hired an evaluation specialist in FY2016 to work with program staff to strategically manage the evaluation of the center's first four years of programming. The evaluation specialist is providing leadership to and oversight of the CGH evaluation portfolio, in addition to assisting with developing performance measures, evaluation questions, survey instruments, and data analysis strategies; managing an evaluation working group; preparing Office of Management and Budget (OMB) packages for approval; and providing scientific and technical writing support for NCI funding opportunities, NCI publications, and CGH website content.

Center for Biomedical Informatics and Information Technology

Support Provided by the Data Science and Information Technology Program

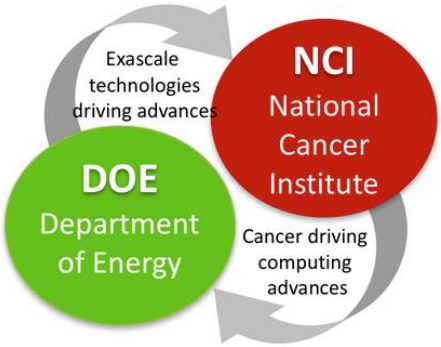
Strategic Initiatives for High-Performance Computing

The Strategic Initiative for High-Performance Computing made significant progress in the past year to advance and accelerate cancer research and clinical applications through expanded use of high-performance computing. The group's efforts were of both tactical and strategic importance, with a focus on developing the capacity within the NCI intramural research community to increasingly employ high-performance computing to address cancer challenges, as well as establishing a longer-term direction and path forward for broader NCI, academic, government, and private industry participation in predictive oncology.

Significant Accomplishments

NCI and Department of Energy Memo of Understanding

After several months of shared effort across NCI, FNLCR, the U.S. Department of Energy, and four national laboratories (Lawrence Livermore National Laboratory, Oak Ridge National Laboratory, Argonne National Laboratory, and Los Alamos National Laboratory), a memo of understanding was signed by Director Lowy and Undersecretary of Energy Orr in June 2016 to support a three-year pilot collaboration to explore three key domains of predictive oncology while defining critical insight into demands for emerging exascale computing capabilities.



The three pilot project domains include:

- Molecular domain pilot – Leveraging the NCI investment to understand the complexities of RAS, which is implicated in nearly 30% of all cancers, the pilot collaboration aims to provide insight into the fundamental molecular biological behavior of RAS-related cancers. Through the use of new experimental data generated at FNLCR, the advanced use of current computing models, and development of new computational approaches targeted for the next generation of exascale computer architectures, the pilot aims to deliver an advanced understanding of RAS-related cancers to identify potential features to target for treatment.
- Preclinical domain pilot – Leveraging the NCI investment to accelerate screening of promising cancer treatments employing a large repository of preclinical cell line and patient-derived xenograft (PDX) models, this pilot effort aims to extend the biophysical model capabilities with computationally predictive models. Integrating data being gathered

during the development of the existing model repositories as well as additional data sources, machine learning and other algorithms are being employed to develop new computational models with an aim to more rapidly focus drug screening efforts on promising treatment candidate drugs and drug combinations.

- Clinical domain pilot – Again leveraging the NCI investment in the SEER registry created by the Division of Cancer Control and Population Studies, this pilot aims to accelerate and broaden the collection, integration, and subsequent analysis of clinical data gathered from real-world clinical experience with cancer patients. High-performance computing capabilities are being employed in the early stage processing of data as well as subsequent analysis and predictive model development of the integrated data.

Public-Private Partnerships

The June 2016 Memo of Understanding between the NCI and the U.S. Department of Energy has been extended through the development of a collaborative Statement of Principles between the NCI, the U.S. DOE, and GlaxoSmithKline to prepare foundations for a sustainable public-private partnership, Accelerating Treatment Opportunities for Medicine (ATOM). The partnership was announced at the June 29 National Cancer Moonshot Summit. Members of the HPC initiative have also contributed to the Cancer Moonshot Initiative through participation in the workforce development committee.

Broadening Participation

Broadening participation and growing the community of experience around the growing use of computational approaches for cancer research and clinical applications has been a central component of the High-Performance Computing Strategic Initiative to expand the workforce and resources required to sustain the computational initiative. Several activities were undertaken in this effort.

Computational Approaches for Cancer Workshop

The group launched a new workshop series on "Computational Approaches for Cancer" at the annual International Conference on High-Performance Computing, Networking, Storage, and Analysis held in November. The initial workshop in 2015 was attended by approximately 80 participants with invited presentations from academia and clinicians as well as forward-looking views shared by Center for Biomedical Informatics and Information Technology (CBIIT) Director Dr. Warren Kibbe, ABCC Director Dr. Jack Collins, and DOE Scientific Advisor Dr. Dimitri Kusnezov. A second workshop has been accepted for the conference in 2016 in Salt Lake City, Utah.

Frontiers of Predictive Oncology and Computing Meeting

Working collaboratively with Intel, the first meeting on Frontiers of Predictive Oncology and Computing was held in July. The meeting was attended by nearly 100 participants from the NCI, DOE, five DOE national laboratories, FDA, DoD, academia, Intel, and other industrial partners with a common interest in exploring the convergence of predictive oncology and computing. The two and one-half day meeting included presenters from industry, academia, and government as well as focused breakout sessions exploring opportunities and challenges for the convergence of predictive oncology and computing in the molecular, preclinical, and clinical domains. A next meeting is being planned for 2017.

Convergence of Predictive Analytics and Big Data

A panel presentation was co-organized for April 2016 at the BioIT World Conference. In this panel, presenters examined current issues and challenges as well as future opportunities for predictive analytics throughout the computational ecosystem, from health learning systems to autonomous processing by sensor arrays.

Organizational Development

Efforts were also underway at a tactical level to better prepare the services and infrastructure that will be needed to support computationally and data-driven cancer research and clinical applications.

- Staffing support for the HPC initiative has grown with the addition of a new HPC Analyst who will work with investigators, developers, bioinformaticians, and computational and data scientists to readily address challenges and develop opportunities for using HPC systems within the NCI and NIH as well as systems available remotely such as those at the DOE Leadership Computing Facilities.
- Long-range plans were developed within CBIIT for several areas, including high-performance computing and cloud computing.
- New services have been developed to ease the movement, sharing, retention, and reuse of valuable experimental and analysis datasets generated within the NCI intramural research community. In addition to expanded use of the Globus Connect endpoints at both CBIIT and FNLCR, a new implementation agnostic data archive service has been developed, employing leading-edge object data storage technologies. RESTful as well as command-line interfaces have been developed to support stable integration into organizational workflows.
- Represented NCI at the Graphics Processing Unit (GPU) Hackathon held at the University of Delaware in May. The Hackathon provided FNLCR participants opportunities to work on key applications side-by-side with experts in the field, gaining valuable insight to bring back to the NCI for use

across a growing number of application areas including deep learning, image processing, and other data-intensive and data science applications.

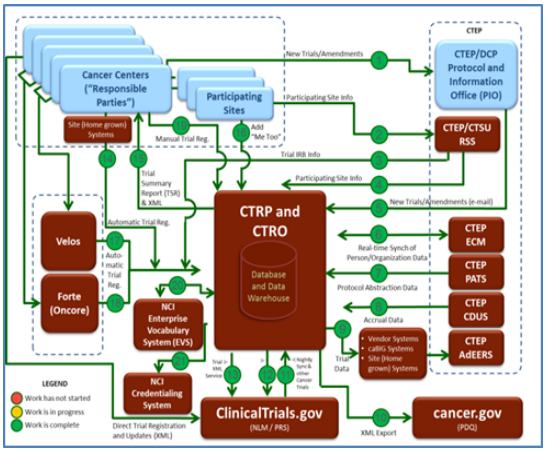
- Commenced programs focused on expanding internal communication and education around opportunities to accelerate cancer research and clinical applications through computational and data science approaches that employ high-performance computing capabilities and services.

The Clinical Trials Reporting Program

The Clinical Trials Reporting Program (CTRP) was established in response to a recommendation from the NCI Clinical Trials Working Group to the National Cancer Advisory Board and was reiterated by the Institute of Medicine's report titled "A National Cancer Clinical Trials System for the 21st Century: Reinvigorating the NCI Cooperative Group Program." CTRP is a comprehensive database of regularly updated information, including accrual, on all NCI-supported clinical trials. This database of the entire NCI portfolio helps identify gaps in clinical research and duplicative studies to facilitate effective clinical trial prioritization and enhances patient accrual to trials by making physicians aware of relevant opportunities for participation in clinical trials (CTRP, 2011).

Leidos Biomedical Research (Leidos Biomed) has been working on the development of this important system for several years in a phased approach. In the current contract year (CY2016), CTRP release 4.3.1 was deployed to production. This version contains a number of enhancements to the suite, including the following:

- The registration application now allows entry of Program Code information. The Data Table 4 like report also includes this information. This was a significant enhancement to the application.
- The interfaces using RESTful services were built with DCP, CTEP, and RSS. This has resulted in systematic transfer of the trial, participating site, and accrual information from these systems to CTRP.



Road Map for CTRP 4.3.1 as of CY2016

The Clinical Trials Reporting Office

The Clinical Trials Reporting Operations (CTRO) team consists of both scientific and protocol data analysts, person/organization curators, clinicaltrials.gov support, and user support specialists who are charged with the arduous task of clinical trial validation, data abstraction, and trial maintenance, which are processed by the CTRP system. This Leidos Biomed team constitutes a critical resource in support of the CTRP operations. While maintaining a high quality of daily trial abstractions and maintenance in the CTRP, the CTRO team also accomplished the following critical tasks during CY2016:

- Successfully processed trials submitted by the CTRP user community within the 10-business-day Service Level Agreement turnaround.
- Successfully abstracted and/or maintained high-profile trials in CTRP, such as the NCI Molecular Analysis for Therapy Choice (MATCH), Lung-MAP, and ALCHEMIST trials.
- Successfully completed an in-depth CTRP data cleanup activity, including obtaining accurate trial status histories for managed trials and trials submitted by the NCI Designated Cancer Centers.
- Assisted with the preparation of the second round of CTRP data clean-up and will be participating in processing the clean-up tasks for the user community throughout mid-to-late CY2016 and into CY2017.
- Continued collaboration with CTEP, CCR, and the cancer centers to obtain participant accruals for their trials in CTRP.
- Continued collaboration with the Office of Communications and Public Liaison (OCPL) to address Cancer.gov-related user inquiries, since the data abstracted by the CTRO is the source of data on cancer.gov.

National Cancer Informatics Program

NCI Molecular Analysis for Therapy Choice (NCI-MATCH). MATCH is a project sponsored by NCI to develop an informatics system for assigning precision medicine to solid tumor or lymphoma patients based on the genetic profile of the patient's tumor. The goals of the project include the following:

- Identify mutations/amplifications/translocations in patient tumor samples to use as eligibility determination.
- Assign patients to relevant agents/regimens.
- Perform tumor biopsies and sequencing at progression to illuminate resistance mechanisms.
- De-identify samples submitted to central labs.
- Provide umbrella protocol for multiple, single-arm Phase II trials.
- Match each molecular subgroup to a targeted agent.
- Use CTEP-Investigational New Drug for protocol template.

- Allow arms to be added or deleted without affecting other arms.
- Hold device discussion with the Center for Devices and Radiological Health (CDRH).
- Focus on single agents (commercial or experimental) initially.
- Consider combinations for targets that have validated combination targeted therapy.
- Establish minimum dose/safety in Phase I trials.
- Submit study to the NCI Central Institutional Review Board (CIRB) for review.

Eligibility

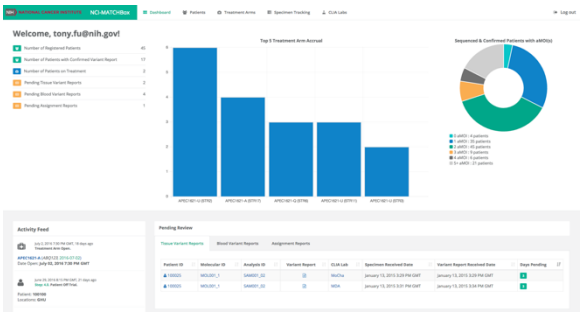
- Solid tumors and lymphomas that have progressed following at least one line of standard therapy.
- Exclude histologies from a given arm of a clinical trial if they are already approved by the FDA for that indication or if the lack of efficacy has been documented.
- The tumor is accessible for biopsy and the patient is willing to undergo biopsy.
- Patient is at least 18 years of age.
- Eastern Cooperative Oncology Group (ECOG) performance status is 0–2.
- Organ function is adequate.

Study Participation

- Eastern Cooperative Oncology Group/American College of Radiology Imaging Network (ECOG-ACRIN) to lead with full cooperation of the Translational Research Advisory Committee NCI Network (NCTN).
- National access through the Cancer Trials Support Unit (CTSU).

The informatics portion of the NCI MATCH trial is designated as NCI-MATCHBox. NCI-MATCHBox integrates data from several external systems at partner organizations with an internal expert system to process genomic, histological, and demographic data according to scientific rules in order to find targeted treatment for the patient.

The NCI-MATCHBox went live in August 2015. To date, over 1,200 patients have been processed through NCI-MATCHBox. With the successful role out of NCI-MATCHBox, a new version of MATCHBOX was commissioned to support pediatric precision medicine clinical trials. The pediatric version utilizes the same expert system built for NCI-MATCH; however, it makes advances in security, workflow flexibility, and MOI detection. Further, the pediatric version of NCI-MATCHBox will be completely deployed in Amazon (AWS) GovCloud and has the ability to compare blood normal to tumoral variants. Currently, PED-MATCH is scheduled to go live in March 2017.



Screenshot of the new dashboard to support PED-MATCH

Clinical and Translational Imaging Informatics Program. The goal of the Clinical and Translational Imaging Informatics Program (CTIIP) is to establish an informatics infrastructure that demonstrates the benefit and feasibility of data interoperability across the three domains: genomics, diagnostic imaging, and digital pathology. This project is divided into several initiatives, which are described below.

Digital Pathology and Integrated Query System

This subproject aims to leverage several open-source and previously NCI-funded activities to provide an open-source, digital pathology image server that can host and serve digital pathology images for any of the major vendors without recoding, which often introduces additional compression artifacts. This has been accomplished by incorporating the OpenSlide library to directly read most of the current whole-slide image formats.

Image annotation is an important aspect of image interpretation and analysis, providing additional metadata and information for use in querying and downstream analysis. The development of a proposal to standardize annotations and markups for pathology images and to harmonize them with the existing standard for annotation of radiology images (annotation and image markup (AIM) language) is part of this subproject. Furthermore, caMicroscope is being expanded to support markup and annotation tools for whole-slide images and to include basic image analysis algorithms utilizing the proposed standard.

This subproject will conclude with the development of a system that queries across pathology data, radiology data, markup, and annotation of each of these image types, along with genomic data. The system will then link this data to patient (human or animal) and outcome data.

Scientific Business Applications Support

The NCI has a significant portfolio of applications and tools needed to manage and maintain the operation of the institute. This includes applications that allow and manage telework agreements for all NCI staff; track Government Furnished Equipment; support new federal employee orientation, internship, and fellowship programs; and manage advocacy groups and other business applications. During this past year, we extended

the telework agreements for all NCI employees and rewrote the underlying system. We also updated the orientation application and revamped the advocacy website. In addition, the team staffed and managed a help desk and provided general application support throughout the NCI.

The Scientific Management Support Team

The National Cancer Institute (NCI) is responsible for issuing and managing more than 10,000 grant actions for more than $3 billion per year in grants-based research. The NCI uses a complex set of processes and procedures to determine which grants should be funded, what should be required for the grant, and what associated information needs to be tracked with that grant. The NCI, as one of the institutes of the NIH, builds on the NIH electronic Research Administration's (eRA) IMPAC II system to track these grants. By extending from the NIH's system, the NCI has built a workflow that supports cancer-specific research and still integrates directly with the NIH grant process. The NCI system, called IMPAC II Extensions, or I2E, follows the complete lifecycle of a grant and provides all of the tools needed for management.

During the past year, the group accomplished several major activities. We developed a new model to massively improve the years audit system, which will ensure that only the proper people have access to grant award and other sensitive information. We updated the Grant Portfolio Management and Tracking System (GPMATS), Paylist, and PFR to support a separate Early-Stage Investigator payline that will allow the NCI to award grants to new researchers with novel approaches. We are rebuilding the Greensheets application to allow much greater reporting and business intelligence, allowing a more detailed meta-analysis of grant activities.

Genetic Data Sharing Policy Tracking System

Recently, the NIH adopted a policy that requires all NIH-funded research generating large-scale genomic data create to maintain a Genomic Data Sharing Plan. These plans will need to be submitted to the NCI in a timely manner. The NCI has a need to track and report on these plans. This team is building an application that will track all GDS plans and their associated artifacts whether the research is funded through an extramural contract, grant, or intramural project. The application will allow the program directors and grant program administrators to manage the entire workflow of the approvals and documents needed to ensure compliance with the NIH-wide policy.

NCI Cloud Pilot Projects

The NCI has contracted three different groups (Seven Bridges Genomics, Broad Institute, and Institute of Systems Biology) to each provide a cloud pilot environment. In addition to developing access to The Cancer Genome Atlas (TCGA) core data sets, each group has developed specific features to help the research community. The NCI needs to independently evaluate each of the pilots, verify that the systems work as designed, ensure that account types behave correctly, and measure performance characteristics.

A Bioinformatics Test plan was developed to evaluate the capabilities of each cloud pilot. The plan will assess the ability of the user to work with both TCGA and custom data; evaluate the performance parameters (accuracy, reproducibility, reliability) of each cloud pilot; and, finally, assess the ability of a user to develop, launch, and monitor analysis pipelines using both pre-built and custom tools. This work is being led by CTOS.

The first Cloud Pilot to be evaluated is the Seven Bridges Genomics (SBG) Cloud Pilot. We have developed a benchmark data-set for a RNA-seq workflow. A standard RNA-seq workflow, adopted by CCBR, has been run on a data-set on Biowulf, the NIH High-Performance Cluster. Output data of this workflow will serve as a benchmark. The SBG infrastructure accepts only dockerized tools. All tools of the RNA-seq workflow have been dockerized using Docker version 1.10.2. All docker image modules have been ported and integrated into the SBG infrastructure, and all modules have been tested successfully. A workflow has been created, within the SBG infrastructure, using the dockerized modules. The cloud-based workflow is currently being evaluated for accuracy (with respect to benchmark results), reproducibility, reliability, and scalability.

Additional workflows to be evaluated include a germ-line variant calling and a somatic variant calling pipeline.

Center for Strategic Scientific Initiatives

Office of the Director

Support Provided by the Data Science and Information Technology Program

High Content Screening of Physical-Based Properties in Biospecimens Phase 2

High Content Screening of Physical-Based Properties in Biospecimens Phase 2, a joint effort between the CSSI, the Division of Cancer Biology's Office of Physical Sciences–Oncology, and the Division of Cancer Treatment and Diagnosis Biorepository and Biospecimen Research Branch, was initiated this year. This subcontracted project is examining the effect of preanalytic variables on the detection and characterization of circulating tumor cells (CTCs) and circulating cell-free DNA (cfDNA) from liquid biopsies from breast cancer patients. The project is employing single-cell genomics and single-cell proteomics methods to evaluate CTCs. The cfDNA is also being genomically characterized by measuring copy-number variations and targeted sequencing. The data from this project will be made

publically available and are expected to help the cancer research and diagnostic communities by providing evidence-based best practices for the collection and storage of CTCs and cfDNA.

Strategic Programs Initiative (SPI)

The SPI within FNLCR was tasked with building a 'Data Coordinating Center' (DCC) for the CSSI. The SysBioCube project, funded by the U.S. Army Center for Environmental Health Research (USACEHR) and supported by CISB, has many overlaps with the DCC requirements and has served as the background in building the technical stack for the DCC. Although different in the architectural details, the initial implementation logic for the DCC is borrowed from SysBioCube.

In the past year, CISB provided technical leadership for building the DCC. In this role, CISB has been actively recruiting developers and validation engineers for the DCC. CISB also worked closely with the subcontractors in clarifying technical details, providing scientific background, and architecting the web interface.

Support Provided by the Clinical Research Directorate

Thrombosis in Cancer Patients

The Thrombosis in Cancer Research Project (YT 14-107NS) is a collaborative effort between the National Cancer Institute (NCI), the Center for Strategic Scientific Initiatives (CSSI), and the National Heart, Lung, and Blood Institute (NHLBI) Division of Blood Disorders and Resources (DBDR) to assess issues related to thrombosis in cancer patients. It is known that the expression and detection of markers in biospecimens can be significantly affected by pre-analytical factors introduced during the process of biospecimen procurement, handling, and storage. The focus of this YT is to conduct pilot research into the impact of pre-analytical variables on key markers that play a role in the relationship between cancer and thrombotic events.

In 2016, the Biospecimen Research Group continued to provide scientific, technical, operational, and project management support to the project. This year, the group established a Leidos Biomed Scientific Advisory Committee comprised of community members and conducted a thorough literature search. Currently, proposals from the community are under review, and an award is expected to be made in August.

Office of Cancer Nanotechnology Research

Support Provided by the Cancer Research Technology Program

Nanotechnology Characterization Laboratory

The Nanotechnology Characterization Laboratory (NCL) has been a leader in the nanomedicine community for more than a decade, having been sought out by over 100 different companies, universities, and various government agencies for expertise in nanoparticle characterization, safety, and more. This year, the NCL received 24 applications to the program and worked on 16 collaborative characterization projects. During the last year, the NCL characterized a total of 39 samples as part of the NCL's Assay Cascade service. To date, the NCL has now characterized more than 360 different nanomedicine products for researchers worldwide.

In addition to this work, the NCL partnered with several pharmaceutical companies for nanotech reformulation and other characterization efforts. These projects were all performed under contractor Collaborative Research and Development Agreements (cCRADA) with Leidos Biomed. NCL worked with AstraZeneca and Amgen on reformulation projects and with Pfizer on the characterization of early nanotechnology platforms. The initial AstraZeneca and Amgen projects have both been completed. The characterization work with Pfizer is ongoing. To date, NCL has characterized two Pfizer formulations and expects several more in the coming months.

The NCL also has been actively involved in a collaborative project with the United States Army's Center for Environmental Health Research (USACEHR). This one-year Interagency Agreement aims to provide a risk hazard assessment of 25 engineered nanomaterials being explored by the Department of Defense. The NCL is providing an extensive physicochemical characterization of the materials; meanwhile, a third partner, Oregon State University, is exploring the biological toxicities of the materials. USACEHR hopes to combine the data generated from both NCL and OSU in computational programs to generate predictive toxicity profiles based on the physicochemical properties of the nanomaterials.

The success of the NCL helped prompt the development of a sister organization in the European Union, the EU-NCL. The EU-NCL involves eight organizations in seven countries and plans to mirror many of NCL's capabilities and organizational aspects. In December 2015, the NCL hosted 13 visiting scientists from the EU-NCL for an intensive two-week training period. The NCL provided lectures on nanomaterial safe handling practices and hands-on laboratory demonstrations and offered many one-on-one sessions for more detailed discussions. The NCL is now assisting the EU-NCL with validation of many of their assays by providing samples for analysis between the two organizations. The validation studies are expected to be completed in the next couple of months.

Office of Cancer Clinical Proteomics Research

Clinical Proteomic Tumor Analysis Consortium Laboratory

Support Provided by the Data Science and Information Technology Program

Clinical Proteomic Technologies for Cancer Antibody Portal

The Reagents Data Portal (http://antibodies.cancer.gov/) continues to serve as a central source of reagents and resources made available by the Clinical Proteomic Technologies for Cancer (CPTC) initiative for the scientific community to support protein/peptide measurement and analysis efforts. This invaluable resource has been developed and maintained to advance proteomics research platforms for the prevention, early detection, and treatment of cancer. The Advanced Biomedical Computing Center (ABCC) Scientific Web Programming Group (SWPG) is continuing to focus on data management and interface improvements to better capture user data and to support future scalability.

The Protein Capture Reagents Program

The Protein Capture Reagents Program (http://proteincapture.org/) is continuing its collaboration between CPTC and the NIH Common Fund to provide low-cost, high-quality renewable affinity reagents for human proteins as a resource for the scientific community. The overall purpose of the program is to create a library of these reagents for public distribution while simultaneously improving upon technologies for generating protein-affinity reagents in an applicable, high-throughput manner. Reagents are produced through a collaborative effort from UniProt, Rutgers, Johns Hopkins University (JHU), the Recombinant Antibody Network (RAN), and the most recent addition, NCI's Antibody Characterization Program.

The ABCC SWPG continues to maintain and develop the site build. As continued in production, this site imports, sanitizes, and manages data from the JHU/CDI laboratory, RAN, Rutgers, UniProt, and NCI's Antibody Characterization Program, bringing all the data together into a single scientific resource. The site features a robust data filter and easy navigation to help users quickly drill into the data, and it builds on the Bootstrap framework, making it mobile-device compatible. While public users have access (http://proteincapture.org/download) to the database Entity-Relationship Diagram (ERD), full database dumps, table definitions, and the latest JSON data import, authenticated user access provides in-depth reporting on the data histories, including targets and shipments by sources. Supporting the "Cloud First" initiative, migration to Amazon Web Services (AWS) has been completed successfully, and the portal now resides in the cloud. Recent additions to the portal include: A new home page that provides a global search, enhanced filtering for quick searching, validation images and files to support the research, a new authentication process to increase security and stability, and numerous utility tools to "watch" the data and files and send alerts if any errors occur from the external sources. Future tasks include collaboration with Linkout and Antibodypedia as well as adding social media integration with Disqus and Twitter.

Support Provided by the Cancer Research Technology Program

Antibody Characterization Laboratory

The Antibody Characterization Laboratory (ACL) continues to produce antibodies (approximately 390 to date) and qualifying data as requested by the Office of Cancer Clinical Proteomics Research. There are presently 140 projects in production. Our current additional focus is to generate antibodies for the specialized application in i-MRM assays for both the RAS program and external groups. A significant effort was undertaken to demonstrate that results from the ACL were in agreement with the Paulovich lab. The ACL screened more than 300 candidate supernatants by Liquid Chromatography tandem Mass Spectrometry in parallel with the Paulovich lab and showed both agreement and selection priority despite using different instrumentation. We also performed a pilot to demonstrate that initial screening could be done using a less labor-intensive method, matrix assisted laser desorption ionization (MALDI). In order to demonstrate potential clinical utility, we have acquired an FDA notified triple quad instrument to verify the assays tested on a clinical grade instrument. All antibody and assay data have been uploaded on the Clinical Proteomic Tumor Analysis Consortium (CPTAC) portals, and antibodies are available from the University of Iowa.

Support Provided by the Clinical Research Directorate

Clinical Proteomic Tumor Analysis Consortium Phase II

The Clinical Proteomic Tumor Analysis Consortium (CPTAC) II project collected and qualified 106 breast, 101 ovarian, 134 colon, and 4 lung cancer cases. The project goal was to collect and qualify 100 cases each for breast, ovarian, and colon. Qualified samples were processed and shipped to the proteomic characterization centers in batches for proteomic analysis. Isolated DNA and RNA were sequenced by the genomic characterization center. Array genotyping, transcriptome sequencing, and micro-RNA sequencing were performed on all samples. Sequencing is complete other than deep-coverage exome sequencing, which was implemented midway through the project in order to achieve better target coverage for subclonal analysis. Deep-coverage exome sequencing was slated for completion in mid-August 2016.

By September 2016, the deep-coverage exome sequencing data was expected to be submitted to the data coordinating center. The remaining samples from the CPTAC II biospecimen core repository (BCR) will be transitioned to the CPTAC III BCR, and the final metadata will be submitted to the data coordinating center.

Clinical Proteomic Tumor Analysis Consortium Triple-Negative Breast Cancer Phase II.V

The goal of the CPTAC Triple-Negative Breast Cancer II.V (TNBC) project is to collect 25 triple-negative breast cancer cases, expecting 15 to qualify. Of the nine prospective cases that were collected, five qualified. Four samples were shipped to the proteomic characterization center for analysis. Two additional prospective cases are on the horizon, with five to six retrospective cases likely to be included.

Sample collection continued until September 30. CPTAC III BCR processed the samples collected/included from July–September using the same protocols/ requirements as CPTAC II BCR. Sequencing will be completed in November, and data will be submitted to the data-coordinating center by the end of 2016.

Clinical Proteomic Tumor Analysis Consortium Phase III

Over the past year, the collection phase of CPTAC III was developed and implemented, and it is now operational. The BCR is fully functional, and four tissue source sites (TSSs) are screening patients and collecting biospecimens from 10 tumor types. The infrastructure for CPTAC III is established, led by the development of the research protocol and standard operating procedures. The Comprehensive Data Resource (CDR) was developed and is actively receiving clinical data submitted by TSSs. The Genomic Characterization Center (GCC) subcontract is in process. At the CDR, 114 cases have been entered, with at least 20 additional cases already collected but not yet entered. The molecular pipeline, including aliquoting and nucleic acid extraction and quality control, will soon be implemented at the BCR.

Center for Cancer Genomics

Support Provided by the Data Science and Information Technology Program

Data Coordinating Center

The Office of Cancer Genomics (OCG) runs several cancer genomics and translation projects, such as:

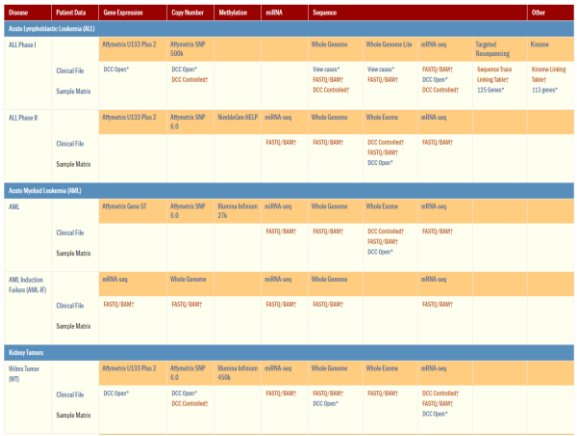
The Therapeutically Applicable Research to Generate Effective Treatments (TARGET) project, which is focused on identifying therapeutic targets as well as prognostic and diagnostic markers in multiple childhood cancers. The initiative includes the study of high-risk acute lymphoblastic leukemia, neuroblastoma (NBL), high-risk and treatment refractory acute myeloid leukemia, osteosarcoma, and kidney tumors (including the high-risk Wilms tumor).

The Cancer Genome Characterization Initiative (CGCI), which supports cutting-edge genomics research on rare cancers. Researchers develop and apply advanced sequencing and other genome-based methods to identify novel genetic abnormalities in tumors. The extensive genetic profiles generated by CGCI may inform better cancer diagnosis and treatment. Focused cancers are Burkitt lymphoma and HIV+ tumor molecular characterization.

The Cancer Target Discovery and Development (CTD^2) program, which works to functionally validate discoveries from large-scale genomic initiatives and advance them toward precision medicine through the efforts of the 13 OCD-supported research teams, called Centers, and open-access data sharing. Through cross-network collaborations, CTD^2 uses innovative bioinformatics and functional biology to mine data to find alterations that potentially influence tumor biology, characterize the functional roles of candidate alterations in cancers, and identify novel approaches that target causative alterations either directly or indirectly.

These programs all contribute to OCG's mission to help identify genomic alterations that offer pathways to novel therapeutic interventions that may lead to more effective cancer treatments.

A Data Coordinating Center (DCC), managed by Leidos Biomed, was established to accept all the data generated by these programs. The DCC has been in place for several years and has updated its functionalities to meet OCG's evolving needs. At a high level of task description, the DCC accepts, quality controls, inventories, processes, stores, and manages data availability. Data includes clinical, genomic, and pathology images. Data availability for public access and controlled access is managed by the DCC and uses a web interface specific to each program. The figures below show the interface to access TARGET and CTD^2 data.



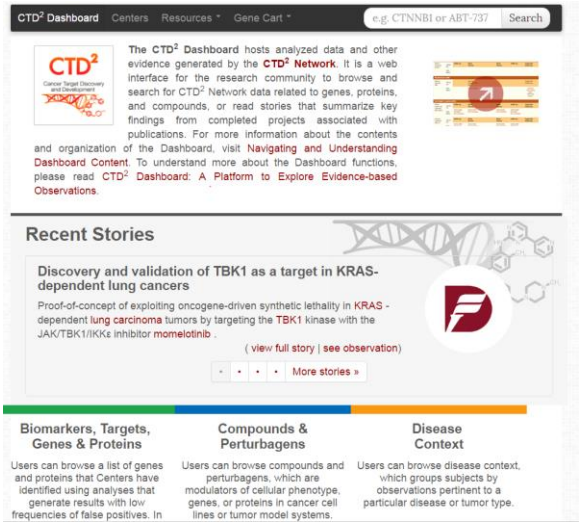
TARGET Data Matrix

CTD² Dashboard

As seen in The TARGET Data Matrix (a data availability matrix for one subset of the programs the DCC manages; top figure), the data range that the DCC handles is extensive, from metadata, exome sequencing, and DNA methylation to mRNA sequencing and clinical data. The CTD² Dashboard (bottom figure) shows a screen shot of the data available for the CTD² program's output.

Unlike many such programs, the submission groups did not have to adhere to any submission standards for data format. Therefore, the DCC team has been very flexible. For example, the team handles the same data type generated on different platforms. The team also manages those instances when groups present the same data types but in different formats. The DCC has built a range of experience, capabilities, processes, and tools to help process the data. A lot of work has also been devoted to managing the logistical elements of the project: tracking the status of sample submission across many different groups, handling sample updates, updating processes/formats to handle new types of information/ assays, and communicating with the submission groups regarding schedule and data definitions. Data are also processed to ensure consistency, quality, and adherence to privacy protection regulations (for example, correct handling of germline variations). Once the data have been thoroughly processed, they are made available using NCI-hosted systems that enable access control; some data are available only to the program's members, whereas data that meet OCG's data release parameters are publically available. Additionally, data prepared by the DCC have already been submitted to the International Cancer Genome Consortium (ICGC).

In addition to all the logistics and bioinformatic activities involved in handling the data that flows into the DCC, the team also supports the website for the CTD² dashboard. The CTD² Dashboard hosts analyzed data and other evidence generated by the CTD² Network. It is a web interface for the research community to browse and search for CTD² Network data related to genes, proteins, and compounds, or read stories that summarize key findings from completed projects associated with publications.



CTD² Dashboard

The TARGET project, from a DCC perspective, is nearing completion. It was a challenging and successful project involving over 70 data sets of clinical and genomic data and 377TB of data submitted to NIH's Sequence Read Archive (SRA).

Recently, the DCC has also extended support for the Gabriella Miller Kids First (GMKF) project, a pediatric-focused project funded by the NIH Common Fund. Also, the DCC has actively supported the Genomic Data Commons (GDC) during GDC development by providing genomic data and user testing. When GDC went live in June 2016, it contained data from three projects: TCGA, TARGET, and CGCI. The latter two projects provided data that had been processed by the DCC.

Future activities of the DCC are to maintain current operations supporting the ongoing data activities of the OCG's genomic data generation programs. In addition to maintaining these ongoing operations, the DCC will maintain its flexibility in adapting to new data and data types needed by the community.

NCI INTRAMURAL

Center for Cancer Research

Support Provided by the Basic Science Program

Human Leukocyte Antigens Immunogenetics Section

Extensive genetic polymorphism is a primary characteristic of the human major histocompatibility complex (MHC) *HLA* class I and class II loci, which encode products that present antigenic peptides to T cells, initiating an adaptive immune response and clearance of foreign material. Variation within these loci is concentrated primarily at positions that alter amino acid sequences and determine specificity for foreign peptides. Apart from their role in antigen presentation to T cells, other characteristics of the *HLA* genes and the molecules they encode have begun to be elucidated. Notably, HLA class I molecules serve as ligands for innate immune receptors, including the killer immunoglobulin-like receptors (KIR) and the leukocyte immunoglobulin-like receptors (LILR) encoded by genes located in the leukocyte receptor complex (LRC). Another more newly defined characteristic of the *HLA* loci is their variable level of mRNA and cell surface expression, which correlates with specific allelic types for some of the loci. This is an important modifier of the strength of the HLA-mediated immune response to cancer and infections, and diversity in expression levels appears to have been selected over time, similar to variation in the peptide-binding groove. A greater understanding of the evolutionary and molecular genetic characteristics of immune response genes is also a key objective of the group. This is an especially important consideration when studying genetic loci composed of multiple homologues that share functional activity, which both the MHC and LRC exemplify, because it is a significant aid in identifying the actual disease locus amongst multiple logical candidates.

The main goal of the HLA Immunogenetics Lab is to understand the genetic basis for resistance or susceptibility to disease conferred by polymorphic immune response loci. The group's approach involves direct testing for genetic effects of polymorphic genes within immune response genes on specific disease outcomes, followed by molecular or cellular biological approaches to understand the basis for the genetic association. These studies have been very beneficial in that they explain and confirm the genetic data and thereby provide solid information for potential use in therapeutic development.

SIGNIFICANT ACHIEVEMENTS

The *HLA-C* locus is distinct relative to *HLA-A* and *HLA-B* in that it is less polymorphic, and it encodes molecules that have lower cell surface expression levels and more extensive interactions with the KIRs expressed by NK cells. Pathogen-driven downregulation of HLA class I molecules on infected cells can result in strongly diminished cytotoxic T lymphocyte (CTL) recognition but also enhanced NK cell-mediated lysis of the infected cell because of the failure of the HLA ligand to bind inhibitory KIRs. The specificity of HIV-1 Nef in downregulating HLA-A and HLA-B molecules, but not HLA-C, has been interpreted as a viral mechanism that subverts adaptive HLA-A and HLA-B–restricted CTL responses while simultaneously protecting infected cells against innate NK cell immunity by recognizing unmodulated HLA-C levels by inhibitory NK cell receptors. The group measured surface expression levels of *HLA* class I on primary CD4+ T cells infected in vitro with primary HIV-1 strains. Unlike the widely studied, laboratory-adapted HIV-1 isolate NL4-3, most primary clones of HIV-1 do in fact downregulate HLA-C to some extent. The group determined, using several primary HIV-1 strains, that the viral Vpu protein is responsible for HLA-C downregulation. Notably, specific naturally occurring amino acid variants in the N-terminal region of Vpu that affect the differential ability to downregulate HLA-C are located within peptides known to bind HLA alleles, suggesting that the ability of HIV-1 to modulate HLA-C could be altered over the course of viral adaptation to certain CTL responses. Differences in HLA-C expression levels have been shown to influence the outcome of HIV-1 infection where higher expression levels associate inversely with viral load in the absence of antiretroviral therapy. Higher HLA-C expression levels also correlate with greater frequencies of HLA-C–associated CTL responses and a higher degree of viral mutation, illustrating the immune pressure that higher HLA-C expression exerts on the virus. That higher HLA-C expression levels associate with viral control is consistent with HIV targeting HLA-C for downregulation. Revision of the prevailing model in which HIV evades both CTL and NK cell immune responses through selective downregulation of *HLA* loci is required and must now take into account mechanisms by which HIV-infected cells evade innate immune cells when HLA-C expression is downregulated.

Previous genome-wide association studies (GWAS) of HIV-1–infected populations have been underpowered to detect common variants with moderate impact on disease outcome and have not assessed the phenotypic variance explained by genome-wide additive effects. Combining the majority of available genome-wide genotyping data in HIV infected populations, about 8 million variants were tested for association with viral load in 6,315 individuals of European ancestry. The strongest signal of association was observed in the *HLA* class I region, which was fully explained by independent effects mapping to five variable amino acid positions in the peptide-binding grooves of the HLA-B and -A proteins. The previously reported strong associations at amino acid positions 97 and 67 in HLA-B were confirmed, and additional signals at position 45 in HLA-B and positions 77 and 95 in HLA-A were

identified. These amino acids are all located in the peptide-binding groove and support the hypothesis that the presentation of specific viral epitopes is critical in determining the efficiency of the CTL response. A second genome-wide significant association signal in the chemokine receptor gene cluster (CCR) on chromosome 3 was also observed. The top single-nucleotide polymorphism (SNP) identified on chromosome 3, rs1015164, is only weakly correlated to *CCR5delta32* ($r2 = 0.03$). This SNP is located within or near an antisense transcribed sequence that overlaps *CCR5* and thus may play a role in regulating its expression. Demonstration of causality of these variants and/or a silencing effect of the antisense transcribed sequence will require functional studies. Overall, the data suggest that common human genetic variation, mostly in the *HLA* and *CCR5* regions, explains 25 percent of the variability in viral load. This study suggests that analyses in non-European populations and of variant classes not assessed by GWAS should be priorities for the field going forward.

Infection with Kaposi's sarcoma-associated herpesvirus (KSHV) is required but not sufficient for development of Kaposi's sarcoma (KS), and the prevalence of KSHV seropositivity far exceeds the incidence of KS. The genes encoding *HLA* and related genes are centrally involved in the immunological response to infectious diseases and thus may affect the risk of developing KSHV infection or KS. The group compared *HLA* class I and *KIR/HLA* ligand frequencies in a two-phase study that included 250 persons with classic KS, 280 KSHV seropositive controls, and 576 KSHV seronegative controls in Italy. This is the first comprehensive study to assess the role of *HLA* and *KIR* on the risk of KSHV seroprevalence and classic KS. The risk of KS was significantly reduced in people with *HLA-A*11:01*, and it was increased for those with *HLA-C*07:01*. *A*11:01* is known to be associated with decreased risk for the Epstein-Barr Virus (EBV)-associated nasopharyngeal carcinoma. Both KSHV and EBV are gamma herpes viruses. This implies that A*11:01 may present herpesvirus-related antigenic epitopes to cytotoxic T lymphocytes, resulting in effective control of the virus in the lytic phase. HLA-C group 1 alleles, which serve as ligands for the inhibitory KIR2DL2 and 3, were significantly associated with protection against KSHV seroprevalence, but with increased risk of KS among KSHV-infected subjects. This KIR/HLA combination has a relatively weak NK cell inhibitory potential relative to that of KIR2DL1 in the presence of its HLA-C group 2 ligand, which is strongly inhibitory. Likewise, the activating KIR3DS1 with HLA-B Bw4 80I was protective against seroprevalence but associated with an increased risk of KS. The hypothesis is that strong NK cell activation protects against KSHV seropositivity but is a risk factor for classic KS after KSHV infection, perhaps because of the known association of KS with inflammation.

Genetic association studies have implicated over 40 genetic loci in the pathogenesis of psoriasis, with the largest signal observed at the *HLA* class I locus for *HLA-C*06:02*. However, recent data also suggest a role for *HLA-B*. Both of these molecules participate in adaptive immunity through antigen presentation, and they also regulate the innate immune response through interaction with KIRs. Previous studies have shown that the presence of activating *KIR3DS1* and *KIR2DS1* is associated with increased susceptibility to psoriasis and psoriatic arthritis, which is consistent with a model wherein expression of activating KIRs increases the risk of developing immune-mediated diseases. Whether decreased expression of inhibitory KIRs is also associated with increased risk of psoriasis has not been fully explored. Cell surface expression of KIR3DL1 varies greatly between alleles, and alleles can be classified into three groups based on their expression levels: KIR3DL1 NULL, KIR3DL1 LOW, and KIR3DL1 HIGH. The ligand for KIR3DL1 is the Bw4 epitope present on several HLA-B molecules. To investigate the association of *KIR3DL1* and *HLA Bw4* with psoriasis, genotyping of *KIR3DL1* and *HLA B* in a cohort of 203 subjects with psoriasis and 111 healthy controls of European descent was carried out. Association testing of the compound *KIR3DL1/Bw4* genotypes revealed a statistically significant association with *3DL1LOW*/*Bw4* and increased risk for psoriasis. Overall, the results suggest that low cell surface expression of KIR3DL1 in the presence of the HLA Bw4 epitope is associated with an increased risk for developing psoriasis, which is consistent with a model whereby a reduction in the inhibitory signal in natural killer or T cells results in a heightened immune cell response.

Differential HLA-C expression levels influence several human diseases, but the mechanisms responsible are incompletely characterized. HLA-C expression levels vary in an allele-specific manner over a range of sevenfold in a pattern that is consistent between African and European Americans and highly reproducible across study groups. Based on the expression value characteristic for each given HLA-C allotype, as previously determined, the group imputed HLA-C expression levels for 228 European individuals from the 1000 Genomes Project (1K) who have previously been typed for *HLA-C*. Imputed HLA-C expression levels were tested as a continuous variable for association with 68,726 SNPs within the MHC using linear regression in order to identify cis-acting variants that may cause (or mark) differential expression of HLA-C. The peak association was centered in the *HLA-C* promoter region, and correcting for population structure did not alter the results. The top signal identified was rs2395471, which is 800 base pairs upstream of the transcription start site. The *A* vs. *G* frequency at rs2395471 was fairly evenly distributed across *HLA-C* alleles. These variants were termed imputed expression quantitative trait loci (impeQTL) to distinguish them from those associating with expression levels of genes that were measured directly. Genotyping of rs2395471 in two independent

cohorts where HLA-C expression levels were measured directly by flow cytometry confirmed the association between this SNP and cell surface expression levels of HLA-C on CD3+ cells. rs2395471 is located in an Oct1 transcription factor consensus binding site motif where the *A* allele is predicted to have higher affinity for Oct1 than the *G* allele. Mobility shift electrophoresis demonstrated that Oct1 binds to both alleles in vitro, but decreased *HLA-C* promoter activity was observed in a luciferase reporter assay for rs2395471G relative to rs2395471A on a fixed promoter background. The rs2395471 variant is predicted to account for up to 36 percent of the explained variation of HLA-C expression. These data strengthen our understanding of HLA-C transcriptional regulation and provide a basis for understanding the potential consequences of manipulating HLA-C expression levels therapeutically.

Molecular Immunology Section

Cytotoxic Cell Studies Group

The Cytotoxic Cell Studies group provides support to the Cancer and Inflammation Program (CIP) in expanding knowledge of the function of the innate immune response and its potential application to the *treatment* of cancer. The characterization of receptors that regulate the activation of natural killer (NK) cells is a major focus of the group. The Cytotoxic Cell Studies group provides expertise in molecular biology in support of the laboratory and program goals. This group has cloned and characterized a large number of murine receptors (Ly49 gene family) that recognize major histocompatibility antigens and control the activation of NK cells. The study of the Ly49 gene family has also led the group to a major discovery in the field of gene regulation: they have found a probabilistic transcriptional switch that controls Ly49 gene activation. This discovery has important implications for controlling stem cell differentiation and may lead to techniques for modulating cell fate in differentiating systems such as bone marrow cultures.

Current research is focused on the human class I MHC receptors KIRs. Probabilistic switches have been identified in the KIR genes, even though this gene family is not related to the murine Ly49 genes. In addition, each KIR gene has been found to contain multiple promoters that are active at different stages of NK development. A classical genetics approach is being used to gain a better understanding of KIR regulation in collaboration with a program project grant headed by Dr. Jeffrey Miller (University of Minnesota). Bone marrow donors have been screened for KIR gene content and expression patterns. Individuals with atypical KIR expression are selected for complete sequencing, and sequence variation predicted to influence expression is then studied in detail. This approach has already revealed a previously unknown upstream promoter element that is required for KIR expression, which was featured in the October 2014 issue of *Genes & Immunity.* Collaborative projects currently under way include: analysis of Ly49 Pro1 function in SLP76-knockout mice in collaboration with Dr. Taku Kambayashi (University of Pennsylvania); functional studies of SNPs implicated in cancer susceptibility, in collaboration with Dr. Michael Dean (DCEG); analysis of RNA-seq data to reveal differences in gene expression in resting vs. activated monocytes, in collaboration with Dr. Dan McVicar (CIP); role of miRNA in the regulation of gene expression, in collaboration with Dr. Ram Savan (University of Washington); investigations of the role of KIR expression in bone marrow transplantation, in collaboration with Dr. Miller.

Significant Achievements

In the past year, the Cytotoxic Cell Studies group has focused efforts on the characterization of novel promoters in the KIR genes together with a complete characterization of polymorphisms in the human HLA-C promoters. The study of a novel upstream KIR promoter has revealed an important role in controlling the tissue specificity and level of expression of different subclasses of KIR genes. The characterization of Pro-I in all of the known KIR genes provided important insights into the expression patterns of KIR, including the observation that the KIR2DL2, KIR2DS2, and KIR2DL3 proteins are the first to appear in reconstituting NK cells after bone marrow transplantation. This work is being conducted in collaboration with the laboratory of Dr. Miller at the University of Minnesota Cancer Center. The Miller laboratory is leading the field in the use of adoptive NK transfer for improving the outcome of bone marrow transplantation. The ability to control the frequency of KIR expression in developing NK cells may improve clinical outcomes. The characterization of the novel intermediate KIR promoters and their role in tissue-specific expression was published in the January 2016 issue of *Genes & Immunity*.

The HLA-C genes are key ligands of KIR, and analysis of allelic variation in HLA-C promoters has demonstrated a role for SNPs in key transcription factor binding sites in tuning the level of HLA-C expression. The study of HLA-C polymorphisms is near completion and will be submitted for publication soon. Further investigation of tissue-specific HLA-C control elements is underway. In addition, several collaborative studies have been completed and will be published soon. First, a change in the properties of the *Ly49* probabilistic switch in SLP76-knockout mice that leads to decreased Ly49 expression was discovered in collaboration with Dr. Kambayashi, and the paper describing the study was recently accepted by *PLoS Biol*. Next, an RNA-seq analysis of mature mouse NK cells has been completed in collaboration with Dr. McVicar and is in press at *Genes Immun*. This study confirmed the immature NK cell specificity of the Pro1 probabilistic switch and provided a complete picture of the transcriptional landscape of the *Ly49* loci. Third, a study on the role of SNPs in the human TET2 gene and their association with prostate cancer in collaboration with Dr. Dean's lab has been completed and

is being revised for publication in *Oncogene*. Finally, a manuscript on the role of a specific SNP in the control of HLA-C levels performed in collaboration with Dr. Mary Carrington will be submitted to *Am J Hum Genet.*

Molecular Immunotherapy Section

The major goal of the Molecular Immunotherapy Section (MITS) is to specifically enhance tumor cell death using chemical, biological, or pharmaceutical agents that sensitize cancer cells to the cytotoxic effects of immunotherapy. Most studies have focused on identifying compounds that sensitize cancer cells to the apoptotic effects of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a pro-apoptotic member of the tumor necrosis factor family of proteins that is produced by various immune cells. The MITS has continued its work on identifying novel compounds that promote apoptosis of cancer cells in response to TRAIL and other death ligands of the TNF family and attempt to determine their molecular mechanisms of action. Within the last year it has become apparent that some of these sensitizing compounds that we have identified can also promote apoptosis in certain cancer cells (particularly melanomas) in response to the viral mimetic poly (I:C). This apoptosis signaling involves the binding of poly (I:C) to a toll-like receptor TLR3. Currently, little is known concerning the molecular basis for the amplification of apoptosis signaling in response to poly (I:C).

Significant Achievements

Sensitization of cancer cells to apoptotic cell death. MITS published the first in vivo evidence that a combination of the proteasome inhibitor bortezomib (Velcade) with an agonist antibody to the TRAIL receptor could provide significant therapeutic benefit over either agent alone by promoting cancer cell apoptosis. Human Genome Sciences carried out a clinical trial of the Velcade/agonist TRAIL receptor antibody combination in patients with advanced multiple myeloma at multiple clinical centers worldwide. More recently, we demonstrated that, with appropriate scheduling and dosing, bortezomib could be combined with adoptive T cell transfer to improve therapeutic benefit in a preclinical mouse model of renal carcinoma (Shanker et al., *Cancer Res*, 2015, 75: 5260 and Pellom et al., *Immunotherapy*, 2015, 7:1011).

In a collaboration between NCI colleagues and Basic Science Program (BSP) scientists in the Molecular Targets Laboratory (MTL), a high-throughput cellular screening (HTS) assay using the human renal carcinoma cell line ACHN was developed to look for novel compounds that could sensitize tumor cells to TRAIL. More than 50,000 pure compounds or natural products were screened using this assay. We identified a number of natural products, purified from extracts by MTL, with the ability to sensitize cancer cells to apoptosis in the presence of TRAIL (Henrich et al., *Apoptosis*, 2012, 17: 79). More recent studies suggest that certain withanolides (derived from medicinal plants) significantly sensitize tumor cells to TRAIL apoptosis both in vitro and in vivo. A very extensive analysis of the molecular signaling pathways involved in TRAIL-mediated apoptosis suggested that the main molecular mechanism of action of withanolide E involved a rapid reduction in the protein levels of the anti-apoptotic protein cFLIP. Interestingly, this reduction of cFLIP involved an increased degradation of the protein rather than any major effects on cFLIP transcription. A patent application was filed covering these findings, and this data was recently published (Henrich el al., *Cell Death Dis*, 2015, 6: e1666). In August 2014, we established a scientific collaboration with Professor Leslie Gunatilaka (Director, University of Arizona Natural Products Center), a medicinal chemist and world expert on withanolides. He has subsequently supplied us with over 80 chemical derivatives based on the withanolide E structure. We have identified derivatives that are four to eightfold more potent than withanolide E in sensitizing cancer cells to TRAIL. This has enabled Structure Activity Relationship (SAR) studies on withanolides. We have found that a particular subset of withanolides, the 17Beta-hydroxywithanolides (17-BHW), are the most active TRAIL sensitizers. Our recent findings suggest that the 17-BHW scaffold can be modified to enhance sensitization of cancer cells to TRAIL-mediated apoptosis. Our group is further testing the anticancer activity of these derivatives in vitro and in vivo. The active 17-BHW are more effective in reducing cFLIP levels in sensitized cancer cells and seem to enhance the assembly of the death-inducing signaling complex (DISC) of proteins critical for the initiation of TRAIL apoptosis. This work is currently being written for publication.

Over the last year, we also made a number of surprising observations when testing active 17-BHWs. First, we observed that active 17-BHWs could also sensitize melanoma cells to apoptosis in response to the viral mimetic poly (I:C). To our knowledge, this is the first demonstration that these natural products can also augment apoptosis signaling by poly (I:C). Additional studies have revealed that initial apoptosis signaling involves the TLR3 receptor for poly (I:C). Furthermore, downstream signaling involved promotion of assembly of a protein complex consisting of FADD, caspase-8, cFLIP, and RIP, a complex very similar to that involved in TRAIL apoptosis signaling. Thus, it is likely that 17-BHWs act on a common downstream signaling pathway to promote apoptosis in response to both TRAIL and poly (I:C). Since poly (I:C) is a well-known immune adjuvant, we propose that a combination of active 17-BHWs and poly (I:C) may provide additional therapeutic benefit over either agent alone in melanoma, not only by promoting cancer cell death but also augmenting the anticancer immune response. Preclinical studies are underway to determine if this occurs. Second, in an attempt to identify the cellular protein targets of active 17-BHWs, Professor Gunatilaka synthesized a number of

derivatives that also contained a biotin group. Surprisingly, one of these biotin derivatives exhibited very little activity in sensitizing renal carcinoma cells to TRAIL or poly (I:C) apoptosis, yet was extremely active in sensitizing certain melanoma cells to both agents. Studies are underway to utilize this biotinylated 17-BHW to identify potential cellular target proteins in melanoma cells, as well as to test its therapeutic efficacy in mouse melanoma models in combination with various immunotherapies.

Active 17-BHWs and specific inhibition of prostate cancer cell growth. One of the most active 17-BHW analogues for apoptosis sensitization obtained from Professor Gunatilaka had originally been identified based on its ability to specifically inhibit the growth of human prostate cancer cells in vitro. We have extended some of these findings using the analogues provide to us by Professor Gunatilaka for a more detailed SAR analysis. The active analogues seem to be causing growth inhibition of these prostate cancer cells by effects on the cell cycle. Some of these studies were carried out by a visiting Ph.D. candidate from Sheffield Hallam University, UK, who spent two months in the laboratory as part of an ongoing collaboration. Recent data confirms the specificity for prostate cancer cells but extends this observation showing that both androgen-responsive and androgen-resistant prostate cancer cell lines are highly sensitive to growth inhibition by the active 17-BHWs.

Hematopoiesis and Stem Cell Biology Section

The major task of the Hematopoiesis and Stem Cell Biology Section (HSCBS) is to define the molecular events that regulate hematopoietic stem cell (HSC) quiescence, survival, self- renewal, and cell fate decisions. The HSCBS efforts are currently focused on defining the physiological function of transcription factors, which are the downstream mediators of signal transduction pathways activated by a cell non-autonomous mechanism from cells in the microenvironment, and cell intrinsic programs in HSCs and their progeny. In addition, we are focused on how individual transcription factors are integrated into wider transcriptional regulatory networks and how combinatorial transcription factor interactions within these networks maintain HSC quiescence, promote self-renewal, and drive lineage-specific gene expression programs. The HSCBS is pursuing these studies to identify novel gene targets and pathways to treat hematopoietic malignancies, myeloproliferative disorders, and/or anemia.

Significant Achievements

The HSCBS has continued its efforts to identify novel transcriptional regulators of HSC proliferation, self-renewal, and differentiation. The HSCBS discovered a novel zinc finger transcription factor, pogo transposable element with zinc finger domain (Pogz), in a screen to identify transcriptional regulators of HSC growth and differentiation. They found that Pogz is an essential gene for embryonic development in a mouse model that lacks Pogz gene expression, as these mice did not survive beyond embryonic day 16. The HSCBS discovered that the expression of transcription factors that regulate erythroid development were greatly reduced in *Pogz-/-* FL, including Bcl11a, which is required to repress fetal globin gene expression. The HSCBS examined globin gene expression and found that fetal globin gene expression was upregulated in *Pogz-/-* FL. The HSCBS discovered that fetal globin genes are expressed in peripheral blood cells (PBC) of mice transplanted with FL HSC from *Pogz-/-*. To confirm these results, the HSCBS transplanted HSC from conditionally targeted Pogz mice, where Pogz is specifically in adult hematopoietic cells (*Mx1-cre;PogzF/F* mice). They discovered that loss of Pogz expression in adult mice results in high levels of fetal hemoglobin in PBC, demonstrating that Pogz is intrinsically required to suppress fetal globin gene expression in adult hematopoietic cells. Furthermore, the HSCBS found that PBC from *Pogz+/-* mice, which show no overt phenotypes, express elevated levels of fetal hemoglobin, suggesting that reducing Pogz levels can increase fetal hemoglobin expression. In conclusion, the HSCBS has provided evidence that regulating Pogz expression might be a novel therapeutic opportunity to treat patients with sickle cell anemia and other hemoglobinopathies.

The HSCB has continued its efforts to define the physiological function of inhibitor of DNA-binding proteins (Id) in normal and malignant hematopoiesis. They found that Id1 is required for normal hematopoietic development, and that mice that lack Id1 show increased HSPC cycling, decreased B Cell and erythroid cell development, and increased myeloid cell development in the bone marrow. Further, they found that Id1 was required for proper microenvironment function, and that niche cells show dysregulated cytokine and chemokine production, which contributed to the observed immunophenotypes. In addition, the HSCBS showed that HSC that lack Id1 exhibit normal hematopoietic development, suggesting that Id1 is not intrinsically required for normal hematopoiesis. However, HSCBS has discovered that HSC that lack Id1 genes show a greatly enhanced ability to self-renew in serial bone marrow transplantation assays. Specifically, *Id1-/-* HSC were able to repopulate lethally irradiated mice after six serial bone marrow transplants, while *Id1+/+* HSC exhaust after three bone marrow transplants. The HSCBS showed that the total number of HSCs was preserved during serial bone marrow transplantation, and that the transplanted HSC show reduced cell cycling, suggesting less proliferation in vivo. In addition, the HSCBS determined that the HSC also showed less DNA damage. Collectively, their results indicate that *Id1-/-* HSC are protected from the proliferative stress of a bone marrow transplant microenvironment. Current studies are focused on understanding what cells/cytokines are responsible for driving the proliferation of HSC, and how *Id1-/-* HSC escape the proliferative stress. In addition, the HSCBS is examining the role of Id1 in the aging of HSC.

Molecular Genetic Epidemiology Section

The objectives of the Molecular Genetic Epidemiology Section (MGES) are to identify causal genetic factors that modify risk for complex and infectious diseases, elucidate the pathophysiological pathways, inform clinical decision-making, and provide new targets for therapeutic intervention. The group has been particularly interested in the genetic basis for health disparities in human diseases, focusing on HIV-associated kidney disease and liver cancer. African Americans with chronic kidney disease (CKD) are four times more likely to develop end-stage renal disease (ESRD) compared to their non-African counterparts, and blacks with untreated HIV have a 50 percent lifetime risk of developing HIV-associated nephropathy, a rapidly progressive form of kidney disease. In a series of collaborative studies, MGES has shown that this propensity is due largely to variant alleles in the *APOL1* that are restricted to people of African ancestry and are rare-to-absent in Asians or Europeans. *APOL1* encodes apolipoprotein L1, a protein that confers resistance to *Trypansoma brucei*, the cause of African trypanosomiasis (sleeping sickness), and is a component of high-density lipid (HDL) particles. In a series of collaborative studies, the group has shown extremely strong associations for two common codon-altering *APOL1* variants (termed G1 and G2) for HIV-associated nephropathy (OR 29), focal segmental glomerusclerosis (OR 19), and ESRD due to hypertension (OR 7). Recent selection has driven these variants to high frequency in West Africa as well as among African Americans, of whom 25 percent and 13 percent, respectively, carry high-risk genotypes.

The MGES is also investigating the role of variation in the APOBEC3 gene family members on HIV disease and cancer. APOBEC3 (A3) cytidine deaminases restrict retroviruses, including HIV, and mobile retro-elements, but they can also hypermutate host ssDNA. Several A3 genes (including A3D, A3G, and A3F) are human intrinsic resistance factors to HIV-1 by causing hypermutation of the nascent cDNA. A3B/A3A are strong endogenous mutagens in several cancers, and *TERT* promoter mutations are among the most common somatic driver mutations in human cancers. However, the relationship of these two genes in cancer has not been investigated. A3B deletion polymorphism, which is also highly stratified in populations, is common (37 percent) in East Asians but rare in Africans and Europeans. The group plans to test for the correlation and interaction of somatic TERT mutations and germline A3B deletion in 276 HCCs of Chinese patients. They are also planning a comprehensive detection of A3 mRNA and proteins in HCC tissues to assess the specific impact of each member of seven A3 genes on HCC cancer. The study will provide evidence for our hypothesis that the mutagenic APOBEC3 proteins contribute to TERT promoter mutation initiation and that the two genes cooperate in tumor initiation.

Significant Achievements

***APOBEC3F* genetic variants restrict HIV-1 disease progression.** In a series of studies, the MGES reported that genetic variants in the A3G, A3B, and CUL5 of the A3-VIf pathway affect HIV-1 disease progression. Although in vitro A3F shows anti-HIV-1 activity and is partially resistant to HIV-1 Vif degradation, it was unknown whether A3F affects HIV-1 disease in vivo. To assess the effect of the *A3F* gene on host susceptibility to HIV acquisition and disease progression, the group performed a genetic association study in 700 HIV-1 seroincident patients that have been followed up with for over 10 years. A common (f=XX) codon-changing variant (p. I231V) was associated with significantly lower set-point viral load, 30 percent slower rate of progression to AIDS, and delayed development of pneumocystis pneumonia (PCP). They further carried out a replication study in the International Collaboration for the Genomics of HIV (ICGH) consisting of more than 10,000 HIV-1 patients worldwide from 25 cohorts and found the consistent association with lower viral load. They have supporting functional evidence that A3F I231V variant influences Vif-mediated A3F degradation. Our results, recently published in *PLoS Genet*, provided the first genetic epidemiological evidence that A3F modulates HIV-1/AIDS disease progression. Currently, drugs are being developed that target the A3G-Vif axis, but our data suggest A3F-Vif pathways may also be excellent druggable targets.

TERT promoter somatic mutations in hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC). Somatic mutational events that confer a growth advantage are required to initiate carcinogenesis. Telomerase reverse transcriptase (TERT) is silent in most normal differentiated cells, allowing telomere length to shorten at each cycle of cell division, which results in cell senescence. However, 90 percent of human cancers have high re-activated TERT activity, leading to unregulated cell division and tumor growth. TERT promoter mutations related to TERT re-activation and initiation of multiple cancers are among the most frequent noncoding somatic mutations in multiple cancers. In a collaboration with Drs. Liu and Guo, Wenzhou University, we investigated the clinical and pathological implications of TERT promoter mutations in hepatitis B virus (HBV)-associated HCC. The MGES sequenced the TERT promoter region for hotspot mutations in HCC tissues and performed immunostaining for TERT protein expression from HBV-associated HCC in 276 Chinese patients. The group reported in *Oncotarget* that 31 percent of HCC patients carried TERT promoter mutations. TERT promoter mutations were more frequent in those with low α-fetoprotein (AFP) serum levels, those of advanced age, and in those lacking HCC family history, but were not correlated with HCC stages and grades.

***APOL1* phenotypic associations and functional consequence.** Because of the very strong associations of APOL1 renal risk variants with kidney disease, the group investigated how variant APOL1 damages the kidney

using a variety of methods, including imaging/cell biology, expression, and explorations of histological phenotypes to address the patterns of injury associated with carriage of two APOL1 risk variants. It has long been hypothesized that reduced glomerular or podocyte numbers were associated with hypertension and chronic kidney disease. Since renal biopsies are rarely, if ever, performed on healthy kidneys, they explored the association of APOL1 with glomerular number in whites and blacks in an autopsy study of black and white Americans who died suddenly from misadventure or cardiac disease using data acquired by researchers at the University of Mississippi Medical Center and Monash University in Australia. They observed that individuals carrying one or two *APOL1* risk variants experienced a loss of nearly 9,000 glomeruli by mid-adulthood, these losses were amplified in the group with body mass indexes above group medians. As glomeruli are lost, the remaining glomeruli tend to enlarge to compensate, and the group found a commitment increase in glomerular volume. These findings indicate that APOL1 are associated with age-related nephron due to decaying glomeruli in early adult life, along with enlargement of the remaining glomeruli. These phenomena might indicate the mechanisms of accentuated susceptibility to APOL1-associated kidney disease in African Americans (published in *J Amer Soc Nephrol*).

Most APOL1 studies have been conducted in persons with kidney disease, but the MGES was also interested in how *APOL1*-associated kidney disease manifests in healthy young adults over their life. In a collaboration with CARDIA investigators, published in *J Amer Soc Nephrol*, the MGES determined that among 3,030 individuals with 20 years' follow-up, *APOL1* high-risk status was associated with loss of kidney function (decline in glomerular filtration rate, but only in individuals with incident albuminuria, a sign of early glomerular injury and a predictor of cardiovascular disease. This was the first study to show that young black adults carrying two *APOL1* risk alleles were significantly more likely to develop albuminuria and decline in glomerular filtration rate.

One-third of African Americans with sporadic focal segmental glomerulosclerosis (FSGS) or HIV-associated nephropathy (HIVAN) do not carry APOL1 renal risk genotypes, suggesting that other *APOL1* or other genetic variants may contribute to kidney disease. To address this question, the MGES sequenced *APOL1* exons in 1,437 Americans of African and European descent, including 464 patients with biopsy-proven FSGS/HIVAN. Testing for association with 33 common and rare variants with FSGS/HIVAN revealed no association independent of strong recessive G1 and G2 effects. Seeking additional variants that might have been under selection by pathogens and could represent candidates for kidney disease risk, they also sequenced an additional 1,112 individuals representing 53 global populations. Except for G1 and G2, none of the seven common codon-altering variants showed evidence of selection or could restore lysis against trypanosomes-causing human African trypanosomiasis. Thus, only APOL1 G1 and G2 confer renal risk, and other common and rare APOL1 missense variants, including the archaic G3 haplotype, do not contribute to sporadic FSGS and HIVAN in the U.S. population. Hence, our study suggests that, in most potential clinical or screening applications, sequencing APOL1 exons is unlikely to provide any more information than genotyping only APOL1 G1 and G2 risk alleles. This study was published in *Kidney Int*.

The MGES has also contributed to a number of collaborative studies that resulted in 19 articles over the review period. The MGES PI is a member of the H3Africa consortium, which is investigating the role of genetics in diseases and is funded by the NIH and Welcome Trust. The laboratory has established collaborations in South Africa to investigate the role of genetic variation in health disparities in liver cancer and in childhood kidney diseases in black Africans compared to their Asian and white counterparts. The group contributed to a study of the role of cyclophilin A (PPIA) in HIV replication, which demonstrated that HIV infection up-regulates PPIA following HIV infection and enhances HIV replication (published in *J Acquir Immune Defic Syndr*). The MGES also contributed to studies on the association of *APOL1* with chronic kidney disease in children with perinatal HIV infection (also published in *J Acquir Immune Defic Syndr*) and an important study that showed that APOL1 risk variants were associated with more severe outcomes in children with nephrotic syndrome. The group is now investigating the mechanism of *APOL1*-associated podocyte injury using imaging techniques with the OMAL group and has successfully demonstrated that APOL1 proteins form homodimers and localize to endosomes. In collaboration with Dr. Andrey Shaw, Washington University, the MGES contributed to a study that sequenced 3,000 genes in FSGS patients that were expressed in the kidney podocyte, which identified a large number of putative rare variants that the MGES is now testing for association with sporadic FSGS.

Computational Structural Biology Section

Continuing advances in experimental techniques and the accumulation of unprecedented genome-scale experimental data allow the Computational Structural Biology Section (CSBS) to address fundamental questions on cellular behavior under physiological conditions and disease. These questions relate to molecular interactions, principles of bimolecular recognition, and mechanisms of signal propagation involving functionally-impaired mutant molecules. Biological function requires that the biomolecules not only interact, but do so in specific ways and be regulated by certain signals and cellular events. Specificity involves distinct atomistic interactions; regulation involves dynamic behavior, which is modulated by the extra- and intra-cellular environment. Comprehension of these dynamics as a function of conditions, such as other binding events, covalent

(e.g., by post-translational modifications) or noncovalent, or mutational events, is essential for grasping the mechanistic underpinnings of molecular—and thus cellular and organismal—function. Perturbations in intra- and intermolecular communications often lead to cellular malfunction and disease. Research at the CSBC seeks to obtain an in-depth grasp of the biophysical principles underlying individual interactions as well as their organization in cellular networks, processes, and mechanisms. It targets protein function and dysfunction in disease and attempts to unravel key factors that could aid drug discovery. In particular, CSBC focuses on cancer and inflammation, aiming to figure out the mechanism of key oncogenic proteins, such as K-Ras4B, their signaling pathways, and pathways that may emerge in drug resistance and thus may conceivably be targeted prophylactically. Recently, in line with CIP and NCI and NIH at large, CSBC has also taken up pathogenic microbiota and their mode of hijacking signaling in the cell.

Significant Achievements

Some examples of the significant achievements of the group are presented below:

- Ras proteins are small GTPases that act as signal transducers between cell surface receptors and several intracellular signaling cascades. They contain highly homologous catalytic domains and flexible C-terminal hypervariable regions (HVRs) that differ across Ras isoforms. KRAS is among the most frequently mutated oncogenes in human tumors. Surprisingly, we found that the C-terminal HVR of K-Ras4B, thought to minimally impact the catalytic domain, directly interacts with the active site of the protein. The interaction is almost 100-fold tighter with the GDP-bound than the GTP-bound protein. HVR binding interferes with Ras-Raf interaction, modulates binding to phospholipids, and slightly slows down nucleotide exchange. The data indicate that, contrary to previously suggested models of K-Ras4B signaling, HVR plays essential roles in regulation of signaling. High affinity binding of short peptide analogs of HVR to K-Ras active sites suggests that targeting this surface with inhibitory synthetic molecules for the therapy of KRAS-dependent tumors is feasible.
- The paradigm for Ras function states that resting GDP-bound Ras is activated by guanine exchange factors (GEFs) catalyzing GDP exchange for GTP. GTP binding induces a conformational change in the Switch I and Switch II regions and exposes the effector interaction site in Ras. Ras effectors, such as Raf kinase, bind Ras-GTP with a higher affinity than Ras-GDP, and this allows initiation of signaling. Signaling proceeds until Ras hydrolyzes GTP to GDP with the help of GTPase-activating proteins (GAPs). Subsequently, the low affinity effector-Ras-GDP complex dissociates, and signaling stops. Importantly, our results suggested that the current model of Ras function is incomplete. They showed that HVR is sequestered by the GDP-bound catalytic domain of K-Ras4B but is released in the GTP-bound state. That is, HVR binding dictates how K-Ras4B is activated, how it is recruited to the plasma membrane, and how K-Ras4B signaling is initiated (and stopped after GTP hydrolysis). The HVR residency on the effector lobe suggests that the HVR may function as a switch releasing or retaining K-Ras4B autoinhibition.
- Our finding that oncogenic mutations in K-Ras4B affect the interaction of the HVR with the active site allowed us to speculate that oncogenic mutations may allosterically modulate HVR-binding affinity. The high affinity interaction of HVR with the active site of K-Ras4B also suggests that targeting this surface with synthetic inhibitors could be feasible. Optimization of compounds that bind at the HVR-catalytic domain interface and promote K-Ras4B autoinhibition could be a potentially promising approach for development of potent inhibitors of so-far undruggable Ras.
- *Is nucleotide exchange sufficient to get K-Ras4B activated*? To signal, oncogenic Ras anchors to the membrane and recruits effectors by exposing its effector lobe. Using nuclear magnetic resonance (NMR) and molecular dynamics (MD) simulations, we observed that in-solution farnesylated K-Ras4B-GDP is predominantly autoinhibited by its HVR, while the GTP-bound state tends to populate an activated, HVR-released state. On the membrane, the catalytic domain takes on multiple orientations, including perpendicular and parallel alignments of the allosteric helices with respect to the membrane normal. In the autoinhibited state, the HVR is sandwiched between the effector lobe and the membrane; in the active state, with the farnesyl anchored into the membrane and the HVR unrestrained, the catalytic domain fluctuates, exposing the effector binding site. Dimerization and clustering could rein the fluctuations, producing more productive pre-organized conformations. Notably, we also observed HVR-autoinhibited K-Ras4B-GTP states that display GDP-bound-like orientations of the helices. We thus proposed that GDP/GTP nucleotide exchange may not be sufficient for K-Ras4B activation; instead, composite mechanisms including HVR sequestration, farnesyl insertion, and orientation/localization of the catalytic domain on the membrane can determine the functional state of K-Ras4B. Taken together, we proposed that, in the GTP-bound K-Ras4B, the HVR-capped effector binding site corresponds to an inactive state. We believe that this HVR autoinhibited conformation, which is highly populated in the GDP-bound states but also populated on the membrane in GTP-bound states, plays a significant role in determining the functional state of K-Ras4B. We suggested a scenario

where the guanine exchange factor (GEF)-catalyzed nucleotide exchange may lead to a GTP-bound inactive state. Binding to the membrane (or an effector) may trigger a population shift toward the active state, exposing the binding site and switching the catalytic domain orientation. For the GTP-bound catalytic domain, we observed two states: active and inactive, with the inactive the more populated state. Oncogenic mutations at G12, G13, and Q61 shifted the equilibrium toward the active state. Thus, the HVR not only anchors K-Ras4B in the membrane, but it also plays a key regulatory role in defining the K-Ras4B signaling state on the membrane. Significantly, the emerging mechanism for K-Ras4B activation advocates that nucleotide exchange is not sufficient, since GTP-bound states may still be inactive. Instead, complex mechanisms and competing catalytic domain/lipid interactions, including HVR sequestration, farnesyl insertion, and orientation and localization of the catalytic domain on the membrane act to determine the functional state of K-Ras4B regardless of the type of nucleotide binding. Notably, even when K-Ras4B-GDP autoinhibition is released, the lipids can still inhibit the effector lobe. Our 'composite model' presents a higher level of complexity of K-Ras4B activation than the current model.

- *Are the dimer structures of active Ras isoforms similar*? This question is significant since Ras can activate its effectors as a monomer; however, as a dimer it promotes Raf's activation as well as MAPK cell signaling. We modeled possible catalytic domain dimer interfaces of membrane-anchored, GTP-bound K-Ras4B and H-Ras and compared their conformations. The active helical dimers formed by the allosteric lobe are isoform-specific: K-Ras4B-GTP favors the α3 and α4 interface; H-Ras-GTP favors α4 and α5. Both isoforms also populate a stable β-sheet dimer interface formed by the effector lobe; a less stable β-sandwich interface is sustained by salt-bridges of the β-sheet side-chains. Raf's high affinity for β-sheet interaction is promoted by the active helical interface. Collectively, the dimer conformations of Ras isoforms are not uniform; instead, the isoform-specific dimers reflect the favored interactions of the hypervariable regions (HVRs) with cell membrane microdomains, biasing the effector binding site orientations—thus isoform binding selectivity. Therefore, we questioned whether the Ras dimer conformations are universal or isoform-specific. This has important potential functional as well as pharmacologic consequences. PRISM, a powerful, knowledge-based structural prediction algorithm, provided four possible dimer interfaces for the catalytic domains of K-Ras4B-GTP and similar ones for H-Ras. Through comprehensive explicit solvent MD simulations of the dimers in different states (on the membrane surface with post-translationally modified HVRs, with parameterized farnesyl (for K-Ras4B and H-Ras), and palmitoyl (H-Ras), as well as in solution), we evaluated the interfaces for K-Ras4B-GTP and H-Ras-GTP. The results show similarities, and—importantly—differences between the isoforms. The K-Ras4B-GTP dimer favors the allosteric lobe dimer interface involving α3 and α4 helices, while the H-Ras-GTP dimer stabilizes the helical interface through the α4 and α5 helices interaction. These allosteric lobe dimer interfaces reflect the active Ras dimer formation, since the effector binding sites are accessible for the Raf associations, promoting Raf dimerization. Both K-Ras4B-GTP and H-Ras-GTP stabilize the effector lobe dimer interface, which has a shifted β-sheet extension. This most stable Ras dimer structure is exactly shared with the Raf-Ras association; thus, it constitutes an inactive dimer interface. We further observed that both K-Ras4B-GTP and H-Ras-GTP form a β-sandwich involving β1, β2, and β3 strands. The inactive Ras dimer interface at the effector lobe also overlaps other effectors' binding sites, thus deterring their conjugation. However, Ras effectors such as Raf and PI3K and Hippo regulator RASSF can easily outcompete Ras for the effector lobe interface, suggesting that the Ras dimer formation with multiple interfaces is dynamic in membrane nanoclusters. Taken together, the varied HVR sequences and prenylated states and environments lead to Ras populating different dimerization states with altered preferred membrane interactions, suggesting that Ras dimerization is highly isoform-specific. We concluded that there is no generic preferred dimer organization for all Ras isoforms. The modes of associations are isoform-dependent, pointing to distinct dimer interface pharmacology.
- Recent landmark discoveries revealed that YAP1 and β-catenin are an integral part of cell-cycle regulation in cells with encoded contact inhibition; in parallel, recent striking reports indicated the ability of overexpressed YAP1 to offset MAPK inhibition. These remarkable findings fit with an increasing body of compelling observations that consistently indicates that overexpression of YAP1, as well as proteins upregulating MYC (such as β-catenin, Notch, Hedgehog, and eIF4E), correspondingly promote proliferation of cells treated with MAPK or PI3K inhibitors. Notably, MAPK/ERK and PI3K/Akt/mTOR pathways are well-established to act at the G1 phase cell-cycle restriction. We propose that the emerging picture from these experimental and clinical data points to oncogenic KRAS—and YAP1 and β-catenin—playing similar roles in cell cycle control in tumor initiation. Thus, our major thesis is that overexpression of YAP1 and β-catenin (or, broadly, c-Myc) is able to rescue tumor cells in Ras drug resistance because they act consecutively in the G1 phase through the G1→S cell-cycle restriction point, just like MAPK/ERK and PI3K/Akt do. We

suggested that this explains why mutations in YAP1 and β-catenin (or in the Hippo and WNT pathways) are often observed in KRAS-driven cancers. Further, the independence and correspondence of the two pathways—MAPK and PI3K as well as YAP1 and c-Myc—also explain why, when combined, they can result in more aggressive tumors. Thus, for example, mutations in APC, a tumor suppressor that plays a critical role in the turnover of cytosolic β-catenin as well as in K-Ras, may collaborate in promoting cancer stem cell phenotypes and in promoting drug resistance. On the other hand, K-RasG12D-driven leukemogenesis, where cell contact plays no role, may not require β-catenin.

- Going forward, the implications of our new understanding could be significant. They argue that, to deter drug resistance in KRAS-driven cancers, MAPK and PI3K/Akt/mTOR and the Hippo and WNT (or other c-Myc promoting pathway combinations) should be co-targeted. Currently, multiple strategies against Ras-driven cancers are investigated; some are broad, while others might lead to K-Ras4B specific therapeutics. Promising, powerful interventions are also tested against mutant Ras pathways proteins, such as Raf, ERK, and PI3K. However, even if successful, drug resistance will inevitably take place. Forecasting alternate pathways that can be involved—and elucidating the reason for this involvement—can be expected to open new horizons. Here we propose blueprints toward this critically-important goal: the four equivalent combinations of the components of the two major pathways through which drug resistance can emerge.
- Working out the critical details—pathway linkages, combinations, and actions, as well as specific proteins to target and toxicity—is an immense challenge. Our model indicates that oncogenic signaling in cancer initiation can be organized into two pathways; it also points to their equivalences in cell-cycle roles and is able to suggest an explanation for the drug resistance. However, we as a community still have a long and arduous road to travel to decipher the cell's immense complexity and treat its oncogenic breakdown.
- TRAF3 is a critical control switch that negatively regulates the activation of the canonical and non-canonical NF-κB pathways, as well as a key protein in antiviral immunity. It is no wonder, therefore, that it is targeted by pathogens in multiple ways. Here, we took steps toward unraveling its mechanism of action in the cell and under viral onslaught on the molecular level. Exploiting available crystal structures and supplementing them by modeling, our structural analysis observes that TRAF3 binds many proteins at the same or partially overlapping binding sites. The signaling output can then be determined by the specific temporal interaction, which results in pro- or anti-proliferative cell output. TRAF3 selection of a specific partner among the many interacting at the same site is influenced by the cell state, and many factors are at play.
- Grasping function in the complex cellular milieu and relating the atomic-scale conformational behavior of molecules to the regulation of the protein in the cell is a formidable aim. At the fundamental level, proteins (and RNA and DNA) exist as ensembles of conformational states and work through dynamic shifts of their conformational distributions. At the cellular level, it presents a challenge because of the intricacy of the cellular network and the heterogeneity of the regulatory mechanisms. Living organisms evolved a winning strategy: merging the fundamental with the cellular needs through 'evolvability.' Evolvability strives to bridge the nuanced functional spectrum based on principles of physics and evolution. Evolvability optimizes and adapts available molecular cellular building blocks and molds them for enhanced and more robust cellular function.
- Viral molecular mimicry is a common way of inhibiting (or activating) the host signaling pathways. Taken together, competitive binding and the evolvability of adaptive viral molecular mimicry appear to be key players in cell function as well as its hijacking by pathogens. We believe that one way to grasp the interplay between pathogen, commensal microbiota, and the human host is to fuse the respective networks, including structural data, which is the basis for in-depth mechanistic understanding.

Epigenetics Section

The epigenome regulates gene expression and thus controls the phenotype of a cell. DNA methylation is one of the major epigenetic modifications that is established early in development and is maintained through replication. Our group has previously identified lymphoid-specific helicase (Lsh) as a regulator of DNA methylation in mammalian cells. Mice with a deletion of Lsh die at birth, and every tissue has compromised DNA methylation pattern. As a consequence, Lsh-/- mice have several stem cell defects, including impaired hematopoiesis, reduced overall growth, and defective germ cell development. Our group hopes to define the molecular mechanisms by which Lsh modulates cytosine methylation patterns during development and to understand how these epigenetic changes relate to chromatin structure.

Significant Achievements

We have previously shown that Lsh can influence the methylation pattern at retroviral sequences and endogenous genes, but the precise role of Lsh in the establishment of DNA methylation at a given site remained unclear. In particular, it is not known whether Lsh, a member of the SNF2 family of chromatin-remodeling proteins, can alter chromatin structure or how this can modulate DNA methylation.

In order to study the molecular function of Lsh on chromatin, we established an in vitro embryonic stem cell (ESC)-based system. DNA methylation levels vary during development and are lowest in the inner cell mass of blastocysts before implantation. After implantation, a wave of de novo DNA methylation occurs and is associated with tissue differentiation. ESCs that differentiate in vitro show a similar wave of de novo methylation and can serve as a suitable model to study the molecular function of Lsh in this process.

We generated Lsh-/- ESCs and found that de novo methylation at several repeat sequences was incomplete in the absence of Lsh and fully restored when Lsh was re-introduced into Lsh-/- ESCs. This indicated that Lsh plays a critical role in the establishment of DNA methylation during cellular differentiation. Furthermore, we found that Lsh is directly associated with those repeat sequences that are undergoing de novo methylation and that the presence of Lsh is required for association of the major DNA methyltransferase 3b with these loci.

When we tested functional domains of Lsh, we discovered that the ATP-binding site of Lsh is required for complete methylation and Dnmt3b association with these repeat target sequences. The ATP binding site is essential for ATP hydrolysis and the chromatin remodeling function of SNF2 factors. Thus, our results indicate that the chromatin remodeling function of Lsh is required for effective DNA methylation. To assess chromatin structure, we applied the nucleosomal occupancy assay, in which we detected lower nucleosomal density in Lsh-/- cells at repeat sequences as compared with wild-type controls. Nucleosomal density was restored to wild-type levels upon re-introduction of Lsh into Lsh-/- cells, indicating that nucleosomal occupancy at repeat sequences depends on the presence of Lsh. Finally, we could demonstrate that nucleosomal density depends on the ATP function of Lsh, indicating that Lsh performs chromatin remodeling at those repeat loci.

Our results suggest that the primary molecular function of Lsh is chromatin remodeling via altering nucleosomal density at loci that are undergoing de novo methylation. Altered nucleosomal occupancy in turn modulates the association of Dnmt3b with target sequences and hence supports de novo methylation. Our results connect two major epigenetic features, chromatin remodeling and DNA methylation, and provide mechanistic insights into the interplay of epigenetic pathways.

Chemistry and Nanotechnology Section

Biophysics Resource Group

The Biophysics Resource Group (BRG) provides operational support to scientific instrumentation and computing resources for several laboratories in the NCI Center for Cancer Research (CCR). This support includes: (1) operation, maintenance, and technical support for all nuclear magnetic resonance (NMR) spectrometers located in the Structural Biophysics Laboratory (SBL), Chemical Biology Laboratory (CBL), and MTL; (2) operational support (through the BRG in the SBL) of a shared-use facility that provides all CCR researchers access to biophysical instrumentation and technologies; (3) laboratory management and operational support for the Protein–Nucleic Acid Interactions laboratory; and (4) management and support of the high-power, dedicated computing facility in SBL, which includes a network of multiprocessor cluster computers, file servers, a backup server, personal workstations, and instrument-connected computers supporting data acquisition, molecular modeling, and structure calculations.

Structural Biophysics & NMR Section

Our research focuses on determining structures of protein–protein, protein–DNA, and protein–RNA complexes by using NMR and X-ray crystallography. In addition, we develop new techniques in order to overcome the current limitations in studying large and complicated macromolecular complexes. One of our research projects is to study a host–pathogen conflict between human APOBEC3G protein (A3G) and HIV-1. We hope to contribute to NCI in their effort to develop new therapeutics for AIDS/HIV. A3G is a single-stranded DNA cytosine deaminase that can restrict HIV-1 infection by mutating the HIV-1 genome. HIV-1 developed a counter defense mechanism by which virion infectivity factor (Vif) leads the degradation of A3G through the ubiquitin-proteasome pathway. Our ultimate goal is to generate small compounds that inhibit the A3G ubiquitination by Vif.

We have determined the structures of two functional domains of A3G, including the VIf-binding domain and the catalytic domain. Since we joined Leidos Biomed/NCI in September 2015, we have been working toward the structure determination of the A3G-Vif E3 ubiquitin ligase complex that will provide epitopes to be targeted by small compounds that inhibit the formation of the complex. The A3G-Vif E3 ubiquitin ligase complex contains six proteins, and therefore it is challenging for any structural study. We have made significant progress in reconstituting the A3G-Vif E3 ligase complex in vitro, which is the first step toward structure determination.

Significant Achievements

- We have regenerated and tested plasmids and other biological resources that are necessary for producing A3G and proteins in the Vif E3 ligase complex.
- We have produced the A3G-Vif E3 ligase complex in vitro using purified proteins, which is necessary for the structure determination and development of an assay for screening of inhibitory compounds.
- We have finished a computational screening for compounds by targeting Vif-binding surfaces of A3G. We have purchased 35 small compounds that had high binding score and solubility. They will be tested by the assay mentioned above.

- We have set up crystal trays for A3G and its complexes by hand as well as using robots. Dr. Alex Wlodawer, molecular crystallography laboratory at NCI, allowed us to use his laboratory space and robots for preparation of crystal trays.
- We have taken NMR spectra of various mutants of A3G in order to characterize their structures and biophysical properties in solution. We are thankful for the availability of the 900MHz NMR at the NIH Bethesda campus.
- Started a collaboration regarding the A3G-Vif E3 ubiquitin ligase complex with Dr. Vinay Pathak in the HIV Dynamics and Replication Program at NCI.

Molecular Targets Group

The Molecular Targets Group (MTG) is organized into three subgroups focusing on: (1) assay development and screening; (2) natural products chemistry; and (3) protein chemistry and molecular biology. All three groups collaborate extensively with CCR investigators to develop and apply assays focused on specific cancer-related targets and/or pathways. The goals of these assays are identification of bioactive molecules through high-throughput screening (HTS) and subsequent characterization of the activities of active compounds. Of particular interest to the group is the identification of novel compounds (and novel activities of known compounds) from natural product extracts obtained from the NCI Natural Products Repository and academic collaborators. A typical work flow starts with the development of a highly reproducible assay compatible with high throughput and use with natural product extracts. This is followed by a screen of pure compound libraries (currently up to approximately 70,000 compounds) and of natural product extracts (greater than 180,000 partially purified samples and approximately 40,000 crude extracts from various sources) for samples able to affect the molecular target or pathway of interest. Natural products chemistry focuses on purification and characterization of active compounds from extracts. The group works directly with the CCR MTL and has more than a dozen currently active collaborations with other CCR laboratories (or sections) or clinical branches along with non-NCI (including international) collaborating labs.

Significant Achievements

For the purposes of this report and because the work of the three subgroups is closely coordinated and highly interactive, accomplishments are combined. A large number of targets are being investigated in the laboratory. At any given time in the last year, 15-20 projects were active in the group. Each subgroup contributed to work focused on the following targets (references noted were co-authored by Leidos Biomed personnel in the MTG):

Antiviral proteins New publications demonstrate significant advances in the development of cyanovirin as a therapeutic (*Plant Cell Rep 2016, 35:1309-19* and *Viruses 2016, 8:158*).

EpCAM (Epithelial cell adhesion molecule assay for liver cancer stem cells (EpCAM+)). EpCAM is a cancer stem cell marker in hepatocellular carcinoma (HCC) and several other cancers. One of the inhibitors of EpCAM(+) cell growth has been further characterized as a wnt/β-catenin inhibitor (*Int J Biol Sci 2016, 12:768-75*).

EWS-Fli1 (Ewing's sarcoma-related transcription factor). Work on increase in cytosolic calcium levels as a mechanism for inhibition of EWS-FLI1 activity by Englerin A has been published (*J Biol Chem 2016, in press*). Development and characterization of a series of mithramycin analogs with improved activity was completed and published (*Clin Cancer Res 2016, in press*).

HIV integrase an assay for inhibition of HIV integrase activity has been developed, and primary screening of the group's pure compound libraries has been completed.

MALT1 (inhibition of a protease implicated in B-cell lymphoma). Purification of active compounds from natural product extracts continues along with biochemical characterization of active compounds. A manuscript describing the assay and its results is in preparation.

NF1 (neurofibromatosis type 1 involved in astrocytoma). Characterization of two related natural products, deguelin and dehydrodeguelin, was completed, allowing publication of the assay and results as well as demonstration of the power of partially purified natural product extracts as a screening resource (*J Nat Prod 2015, 78:2776-81*).

PAX3/FOXO1 (fusion transcription factor involved in rhabdomyosarcoma). Primary screening of pure compounds and prefractionated extracts is complete and hits provided to collaborator for confirmation in a secondary gene expression assay looking at reversal of the PAX3-FOXO1 gene expression signature.

p38 (non-canonical activation pathway for p38 in T cells). A series of synthetic compounds with novel inhibitory activity has been characterized and published along with description of the assay (*J Biomol Screen 2016, 21:277-89.*). Bioassay guided fractionation for natural products discovery is ongoing and progressing towards isolation and characterization of active pure natural products.

P300-Hif1 (protein–protein interaction required for hypoxic response). Natural product inhibitors of this interaction continue to be purified and characterized (*J Am Chem Soc 2015, 137:5569-75*).

Pdcd4 (tumor suppressor protein). More novel stabilizers of Pdcd4 have been identified and published (*PLoS One 2016, 11:e0151643*) as a result of this international collaboration.

TDP1 assay (inhibition of phosphodiesterase activity). A novel 42 amino acid inhibitory peptide was isolated from a marine sponge, and its amino acid sequence was determined (tandem mass spec, Edman

degradation). Peptide will be produced in *E. coli* for crystallographic studies (in collaboration with Macromolecular Crystallography Laboratory).

TDP2 assay (inhibition of phosphodiesterase activity). Primary screening of pure compound libraries has been completed. Active compounds were confirmed, including exclusion of those that hit in other biochemical assays (p38 and MALT), yielding 97 compounds now being further assessed with our collaborators (DTB). Screening of natural product extracts is ongoing.

Ras-Raf (inhibition of dimerization). Development of a novel BRET-based assay has been completed along with primary screening of the entire MTL pure compound and natural product libraries. Confirmation, assessment of potency, and subsequent characterization of identified compounds has begun.

SUMO (Small Ubiquitin-like Modifier). Purification of active compounds from natural product extracts continues along with biochemical characterization of active compounds.

TRAIL (TNF-related apoptosis-inducing ligand). A new series of natural product inhibitors of protein synthesis initiation have been identified as TRAIL sensitizers and are being characterized.

Yeast Chemical Genomics Novel insights into cycloheximide's mechanism of action as an inhibitor of actin cytoskeleton dynamics via inhibition of the RhoA GTPase have been published (*J Cell Biochem 2016, in press*), demonstrating the power of this technique.

Other support activities The group has been very active in supporting continued expansion and application of new MTL screening libraries. As noted above, the group has been able to convincingly demonstrate the utility of partial purification of extracts as an important strategy. In addition, in an international collaboration, several novel compounds have been characterized from Amazonian plant fungi (*Nat Prod Commun 2015, 10:1649-54* and *J Nat Prod 2015, 78:3005-10*). The group also continues to participate in the evaluation of potential new MTL projects (four formal project proposals and multiple informal discussions in the last year).

Biomolecular Informatics Group

Scientists of the Biomolecular Informatics Group (BMIG) are involved in computationally characterizing RNA, DNA, proteins, small molecules, and their interactions. Areas of expertise include the computational design of novel RNA nanoscale structures, RNA secondary structure prediction, characterization of protein–ligand interactions, virtual ligand screening, and computational analysis of antibody sequences and structures, as well as techniques related to the design and characterization of RNA and DNA nanoparticles. One BMIG scientist is involved in the experimental testing of de novo–designed RNAs and their delivery formulations. This research supports the groups of Drs. Bruce A. Shapiro and Marc Nicklaus (CCR).

SIGNIFICANT ACHIEVEMENTS

Development of RNA Logic Elements: RNA Switches. The mechanism of RNA interference (RNAi) is a cellular defense mechanism that can be repurposed to down-regulate target genes. The RNAi pathway can be activated by delivering designed RNA duplexes. This capability allows to target oncogenes and has many potential applications for cancer therapy. An important extension of this approach is, however, to ensure that the knockdown of a target gene is only in effect in targeted cells. In other words, there is a need for conditional molecular therapeutics that preferentially function in defined cellular milieu ranges. Together with CCR researchers, we designed and characterized a novel RNA logic element that downregulates a target gene conditional to the presence of an mRNA of a different gene that acts as a trigger and biomarker. This construct, called RNA switch, contains a functional duplex region that is initially in an inactive conformation. RNAs containing specific sequence motifs can act as a trigger, leading to a re-association of the RNA switch and the release of the programmed functionality. We programmed an RNA switch that is conditional to the cancer biomarker gene Connective Tissue Growth Factor (CTGF). The presence of an oncogene within a cell (in the form of its mRNA) can therefore be converted into the downregulation of some other protein needed by the diseased cell, thereby achieving a conditional therapeutic approach. The validity of this novel concept was recently published by us (*Nano Lett* 2016 16(3):1726-35).

Comprehensive Characterization of RNA/DNA Hybrid Designs. Unprotected RNA nanostructures can be subject to nuclease degradation. Nucleic acid structures consisting of duplexes containing both an RNA and DNA strand (RNA/DNA hybrids) are more resistant with respect to nuclease degradation. RNA/DNA hybrids may lack, however, some functionality such as the capability of activating the RNA interference pathway. In order to utilize the advantages of the stability of RNA/DNA hybrids and the functionality of RNA nanostructures, we designed pairs of RNA/DNA hybrids that can, after delivery to target cells, re-associate into functional RNA/RNA and DNA/DNA nanostructures. The functionality restoration is then conditional to the simultaneous presence of the two hybrids in the same cell and their re-association. The process of re-association is initiated by the utilization of DNA toeholds within the hybrids that can drive this process. Additional control can therefore be implemented through the modulation of the toehold length and GC content. The dependency of the re-association reaction on the properties of the toehold regions was experimentally assessed. The measurements match the computational prediction from a program developed within the laboratory, HyperFold, as was recently reported (*Nano Lett* 2016 16(3):1726-35). We explored another design strategy by utilizing RNA-based toeholds as opposed to DNA toeholds. The validity of this concept was tested computationally and experimentally.

This new approach allows us to use toeholds that are simpler to design and leads to smaller nanoparticles with higher yields (*Nano Lett* 2016 16(3):1746-53).

Cellular Delivery of Functional RNA. For the aforementioned nucleic acid constructs to perform their task, they need to be properly delivered to cells. A substantial effort consists, therefore, in the development of molecular delivery agents that facilitate the cellular delivery of nucleic acid constructs. They have to protect the RNA nanoparticle while in circulation to prevent their degradation and facilitate their penetration within diseased cells. We are currently exploring peptide-based and polymer-based carriers. We explored lipid-based carriers with different chemistries and evaluated the parameters that are important for their enhanced functionality as delivery agents. We published reports last year on the potential of Bolaamphiphiles (*J Control Release* 2015 213:142-151) and oxime–ether lipids (*Nanomedicine (Lond)* 2015 24:1-14) as delivery agents. The study on bolaamphiphiles also contains substantial work corresponding to the computational prediction of interactions between RNAs and the delivery agent. The performed research demonstrated that there are multiple options for the cellular delivery of RNA and RNA/DNA nanoparticles in cell culture and mouse models.

Cataloging of RNA Ring Structures. A variety of published RNA nanostructures corresponds to ring-like structures. We previously designed, characterized, and published examples corresponding to RNA triangles, squares, pentagons, and hexagons. Some of these RNA nanoparticles were conjugated with functional RNAi-activating duplex regions or with fluorescent beacons. These RNA ring structures were designed by utilizing multiple copies of RNA structural motifs that correspond to the corners of the RNA-based polygons. It needs to be acknowledged that not all structural motifs correspond to closed-ring structures. This leaves the molecular designer with the difficult task of choosing and computationally manipulating an RNA 3-D motif that will lead to the envisioned target 3-D structure of the RNA nanoparticle. We solved this problem by computationally iterating through more than a thousand motifs obtained from our previously created database of 3-D RNA motifs called RNAJunction. For each chosen motif (or combination of two motifs), our NanoTiler software attempts to build an RNA ring structure. Successful cases were stored, annotated, and made publicly available in form of the Ring Catalog. Together with CCR researchers, we showed that an example obtained from this catalog also forms experimentally. This corresponds to a first example of a new paradigm of a "design by catalog" where a designer of an RNA structure chooses a template for an envisioned structure from a catalog of pre-computed scaffolds (*Methods* 2016 103:128-37).

Basic Research Section

Retroviral-Biochemistry Group

The Retroviral-Biochemistry Group (RBG) supports research of the Reverse Transcriptase (RT) Biochemistry Section and the Model Development Section at the Basic Research Laboratory (BRL).

RBG explores nucleic acid–based strategies to investigate conserved structures in the retroviral genome, mutations, and host–virus interactions as possible targets for new antiretroviral drugs. This work is complemented by chemical biology approaches to identify small molecules that interact with and antagonize cellular and viral regulatory RNAs.

The group also supports efforts to mutagenize, purify, and analyze key retroviral proteins to aid in drug development. The RBG Model Development Section (MDS) seeks to translate basic research findings into model systems that more faithfully mimic HIV infection in vivo in order to address questions on viral transmission, viral pathogenesis, and the contributions of the host immune system in these processes.

Significant Achievements

RGB improved methods to generate large amounts of highly purified, fluorescently labeled RNAs as well as methods for screening small molecule microarrays (SMMs) to identify novel therapeutic RNA-binding small molecules.

Macromolecular Crystallography Group

Significant Achievements

Validation of protein stereochemistry. The Protein Data Bank contains more than 90,000 atomic models of macromolecular structures and is an immensely valuable resource for structural biology, bioinformatics, drug development, and other branches of biomedical sciences. The validity of all models of various proteins and nucleic acids available in the PDB has, therefore, the utmost importance. All atomic models submitted to the PDB undergo several validation routines, where their geometry and stereochemistry are compared to the target standards, formulated on the basis of the known small molecular structures and atomic-resolution crystal structures of proteins. These geometrical standards, used in all crystallographic and NMR refinement and validation software, are available in the library of target values created in the 1990s by Engh and Huber. However, since the time when this library was formulated, many more very accurate atomic models of macromolecules became available. The detailed scrutiny, based on the large number of presently available structures, revealed that certain stereochemical target values appear to be inaccurate and require adjustments. One of the specialties of the Macromolecular Crystallography Groups located at the synchrotron site at the Argonne National Laboratory is the crystallographic work at very high resolution of

diffraction data, and it was decided that the efforts towards improving the geometric target libraries is an appropriate research direction.

The imidazole moiety of histidine can be protonated on either or both of its two nitrogen atoms. Since histidine is often a member of the active sites of various enzymes, it is important to know the protonation state of this amino acid. However, the current target library does not differentiate between bond lengths and angles of variously protonated histidines. The advanced statistical exploration of a large number of atomic resolution crystal structures led to formulation of two functions, his1 and his2, based on two internal bond lengths and two bond angles within the imidazole group that equivocally determine the protonation state of this amino acid on the basis of its geometry.

The Engh and Huber library assumes that the guanidinium group of the amino acid arginine is symmetrical with respect to its internal bond angles around the central carbon atom. Inspection of the PDB validation protocols of a number of protein crystal structures suggested that this group is in fact not symmetrical. The statistical analysis based on a large number of atomic resolution crystal structures of proteins and small structures containing guanidinum moiety permitted to formulate the improved target values of the bond angles, differing by 1.5 degrees from the previously suggested values. The new targets will be incorporated into the improved stereochemical target libraries used in structural crystallography and NMR practice.

Novel noninvasive photosensitizer KillerOrange. Photosensitizers are the chromophores that generate reactive oxygen species (ROS) upon light irradiation. Until 2006, all known photosensitizers have been chemical compounds introduced into living systems exogenously. GFP-like red fluorescent protein KillerRed was the first genetically encoded photosensitizer that could be directly expressed by target cells. Upon green or orange (530–590 nm) light irradiation, KillerRed generates ROS that damage the neighboring molecules. Only four such photosensitizers are known to date: GFP-like proteins KillerRed (λex/λem 585/610 nm) and its monomeric variant SuperNova (λex/λem 579/610 nm), and FMN-binding proteins miniSOG (λex 458 and 473 nm, λem 500 and 528 nm) and Pp2FbFP L30M (λex 448 and 475 nm, λem 495 and 523 nm). Genetically encoded photosensitizers are a promising optogenetic tool for light-induced production of reactive oxygen species at desired locations within cells in vitro or whole body in vivo, resulting in controlled elimination of specific cell populations, target protein inactivation, DNA damage, etc.

To further expand the toolkit of available phototoxic proteins, we have very recently come up with a blue-shifted KillerRed variant carrying tryptophan-based chromophore (substitutions: Gly3Cys, Tyr66Trp, Asp113Ser, Asn145Ser, Phe177Leu, Tyr221His, Glu236Gln) named KillerOrange. We also constructed monomeric mKillerOrange by introduction of the single Tyr66Trp substitution in SuperNova, a monomeric variant of KillerRed.

The absorbance spectra of KillerOrange and mKillerOrange possess two overlapped bands with maxima at approximately 455 nm and 514 nm. Excitation at these wavelengths produces weak cyan (λem approximately 480 nm) and bright orange (λem approximately 555 nm) fluorescence, respectively. Most likely, the shorter and longer wavelength forms correspond to the CFP-like and mHoneyDew-like chromophores, respectively. Unlike KillerRed, which is toxic under green/orange light illumination, KillerOrange develops phototoxicity under blue/cyan light. The new orange variants expand the palette of genetically encoded photosensitizers, and in combination with KillerRed, they would make a useful pair for independent simultaneous control of two cell populations.

Structural analysis of photoconvertible fluorescent protein DendFP. Phototransformable fluorescent proteins (PTFPs) form a distinct class of FPs, the spectroscopic properties of which can be controlled by light. PTFPs comprise three main groups: photoactivatable fluorescent proteins (PAFPs), photoconvertible fluorescent proteins (PCFPs), and reversibly switchable fluorescent proteins (RSFPs). PAFPs are proteins that undergo an irreversible, light-induced activation from a nonfluorescent state to a fluorescent state. PCFPs, on the other hand, exhibit an irreversible conversion between two fluorescent states, whereas RSFPs can be photoswitched back and forth between a fluorescent ON state and a nonfluorescent OFF state. The phototransformations of PAFPs and PCFPs involve covalent modifications of the FPs, while RSFPs undergo only conformational rearrangements of the chromophore and its immediate environment.

Because of the ability of PTFPs to change their fluorescence upon exposure to light of a specific wavelength, they have become essential marker tools for live-cell optical imaging with super-resolution. PAFPs are ideal for regional optical marking in pulse-chase experiments on live cells and tissues. PSFPs can be used in patterned illumination microscopy (for example, RESOLFT), which requires markers that are capable of enduring multiple cycles of reversible photoactivation. Controlled phototransformation of PCFPs enables their use in advanced imaging with resolution beyond the diffraction limit of light. PCFPs are excellent markers for localization-based super-resolution microscopy (for example, PALM).

The fluorescent protein from *Dendronephthya sp.* (DendFP) is a member of the Kaede-like group of photoconvertible fluorescent proteins with a His62-Tyr63-Gly64 chromophore-forming sequence. Upon irradiation with UV and blue light, the fluorescence of DendFP irreversibly changes from green (506 nm) to red (578 nm). The photoconversion is accompanied by cleavage of the peptide backbone at the Cα—N bond of His62 and the formation of a terminal carboxamide group

at the preceding Leu61. The resulting double Cα=Cβ bond in His62 extends the conjugation of the chromophore π system to include imidazole, providing the red fluorescence.

We have solved 3-D structures of native green and photoconverted red forms of DendFP at 1.81 and 2.14 Å resolution, respectively. The structure-based mutagenesis of DendFP revealed an important role of positions 142 and 193: replacement of the original Ser142 and His193 caused a moderate red shift in the fluorescence and a considerable increase in the photoconversion rate. It was also demonstrated that hydrogen bonding of the chromophore to the Gln116 and Ser105 cluster is crucial for variation of the photoconversion rate. These results can be used to engineer improved biomarkers for super-resolution microscopy.

Structural analysis of β-prism lectin from *Colocasia esculenta.* Lectins are omnipresent in animals, plants, and microorganisms that persist as a distinct group of glyco-binding proteins able to agglutinate cells and/or precipitate glucoconjugates. They assist as recognition molecules within a cell, between cells, or between organisms. Plant lectins have many biological and industrial applications. Based on sequence similarity, plant lectins are often divided in several subgroups of structurally and evolutionarily related proteins. The most important plant lectin families are: (1) amaranthins, (2) lectins with a Nictaba domain, (3) chitin-binding lectins, (4) *Galanthus nivalis* agglutinin (GNA)-related lectins, (5) the jacalin-related lectins, (6) the legume lectins and (7) lectins with ricin-B domains. Among these, the GNA-related lectin family has received increasing attention due to its high potential with antitumor, antiviral, and insecticidal properties.

Taro (*Colocasia esculenta*) is a tuberous plant belonging to the family Araceae. Lectins from taro play various important roles in plant defense with insecticidal activities and are characterized to some extent as anticancer and antiviral proteins. We performed purification, crystallization, and structural analysis of the β-Prism; *C. esculenta* lectin. Biochemical characterization of the *C. esculenta* lectin is on the way.

Structural studies of a serine protease inhibitor from *Bauhinia bauhinioides* (BbKI). Plant-derived Kunitz-type inhibitors have been studied very extensively. Their inhibitory properties have been determined for a number of blood-clotting enzymes such as plasma kallikrein, factor XIIa, factor Xa, and thrombin for enzymes involved in digestive processes such as trypsin and chymotrypsin, and for enzymes involved in inflammatory processes such as elastase.

BbKI was isolated from the seeds of Bauhinia bauhinioides. This 18 kDa protein belongs to the Kunitz family of plant inhibitors. BbKI is not active on cysteine proteases but inhibits bovine trypsin, human plasma kallikrein, and plasmin. BbKI is currently the only known inhibitor isolated from plants that inhibits tissue kallikrein in addition to plasma kallikrein. Because of the highly restricted specificity of blood coagulation enzymes, it is easy to understand why so few plant inhibitors are able to block their activities. As is the case for digestive enzymes such as trypsin and chymotrypsin, the enzymes of blood coagulation are serine proteases. However, unlike trypsin, the proteases of the coagulation cascade have acquired a high degree of specificity during the course of evolution, since they cleave only a limited number of peptide bonds involving basic amino acid residues. BbKI (or its recombinant form rBbKI) has become an attractive molecule for studying pathological models of the circulatory system, since this protein acts on plasma kallikren and plasmin, two enzymes involved in coagulation, fibrinolysis, and inflammation. Other important aspects of the inhibitor have been reported by Brito et al. (2014), who found that BbKI may prolong the formation of blood clots in vitro and that it exhibits antithrombotic activity in venous and arterial thrombosis in vivo models. Mutagenesis indicated that residue R64 of BbKI plays an important role in plasma kallikren, since mutant R64A of BbKI is a much weaker inhibitor. The structural studies of BbKI at 1.4 Å and modeling of plasma kallikren binding to BbKI showed that, in order for BbKI to bind kallikren tightly, the reactive loop containing Arg64 needs to undergo a structural change. The ongoing study of BbKi aims to determine the structures of complexes of BbKi with its target proteases to gain the details of their interactions and identify the residues responsible for the specificity. Crystallization of complex of BbKI and trypsin has yielded some hits during initial screening, and optimization is underway. A complex of BbKI and human plasma kallikren will be the next target.

Structure and functional properties of the active form of the proteolytic complex, ClpP1P2, from Mycobacterium tuberculosis. The ClpP protease complex and its regulatory ATPases, ClpC1 and ClpX, in Mycobacterium tuberculosis (Mtb) are essential and therefore promising drug targets. The Mtb ClpP protease consists of two heptameric rings, one composed of ClpP1 and the other of ClpP2 subunits. Formation of the enzymatically active ClpP1P2 complex requires binding of N-blocked dipeptide activators. We have found a new potent activator (benzoylleucine-leucine (Bz-LL)) that binds with higher affinity and promotes three to fourfold higher peptidase activity than previous activators. Bz-LL-activated ClpP1P2 specifically stimulates the ATPase activity of Mtb ClpC1 and ClpX. The ClpC1P1P2 and ClpXP1P2 complexes exhibit two to threefold enhanced ATPase activity, peptide cleavage, and ATP-dependent protein degradation. The crystal structure of ClpP1P2 with bound Bz-LL was determined at a resolution of 3.07 Å and with benzyloxy carbonyl (Z)-LL bound at 2.9 Å. Bz-LL was present in all 14 active sites, whereas Z-LL density was not resolved. Surprisingly, Bz-LL adopts opposite orientations in ClpP1 and ClpP2. In ClpP1, Bz-LL binds with the C-terminal leucine-side chain in the S1 pocket. One carboxyterminal oxygen is close to the catalytic serine, while the other contacts backbone amides in the oxyanion hole. In ClpP2, Bz-LL binds with the benzoyl group in the S1 pocket and the

peptide hydrogen bonded between parallel β-strands. The ClpP2 axial loops are extended, forming an open axial channel as has been observed with bound ADEP antibiotics. Thus, occupancy of the active sites of ClpP allosterically alters sites on the surfaces, thereby affecting the association of ClpP1 and ClpP2 rings, interactions with regulatory ATPases, and entry of protein substrates.

Structure of RC1339/APRc from Rickettsia conorii, a retropepsin-like aspartic protease. The crystal structures of two constructs of RC1339/APRc from Rickettsia conorii, consisting of residues 105–231 or 110–231 followed by a His tag, have been determined in three different crystal forms. As predicted, the fold of a monomer of APRc resembles one-half of the mandatory homodimer of retroviral pepsin-like aspartic proteases (retropepsins), but the quaternary structure of the dimer of APRc differs from that of the canonical retropepsins. The observed dimer is most likely an artifact of the expression and/or crystallization conditions, since it cannot support the previously reported enzymatic activity of this bacterial aspartic protease. However, the fold of the core of each monomer is very closely related to the fold of retropepsins from a variety of retroviruses and to a single domain of pepsin-like eukaryotic enzymes, and may represent a putative common ancestor of monomeric and dimeric aspartic proteases.

Development of inhibitors of human tyrosyl DNA phosphodiesterase 1 (TDP1). DNA topoisomerases regulate DNA topology by transiently cleaving the DNA backbone to remove DNA supercoiling, unlinking post-replication catenanes and resolving DNA knots. DNA cleavage is generated by the covalent attachment of the catalytic tyrosine of the topoisomerase to the end of the broken DNA. DNA topoisomerases are categorized as type 1 or 2, depending on whether they cleave one or both strands of DNA, respectively. Inhibitors of topoisomerases 1 and 2 (Top1 and Top2) that generate stalled topoisomerase cleavage complexes are among the most widely used anti-cancer agents. Tyrosyl-DNA phosphodiesterases are critical for repairing topoisomerase cleavage complexes. Tyrosyl-DNA phosphodiesterase 1 (TDP1) is a Top1 cleavage complex repair enzyme recently recognized as a pharmacological target for cancer treatment. TDP1 repairs DNA lesions created by the trapping of Top1 following treatment by the camptothecin derivatives topotecan and irinotecan. It does so by hydrolyzing the covalent bond between the Top1 catalytic tyrosine and the 3′ end of the DNA. Polynucleotide kinase phosphatase (PNKP) then hydrolyzes the 3′-phosphate and installs a phosphate on the 5′-end at the other side of the break, after which DNA ligase III reseals the DNA. TDP1 function is not limited to the repair of Top1 cleavage complexes, however, as it can also serve as a backup pathway for the repair of DNA lesions created by the trapping of Top2 on DNA. TDP1 can also remove 3′-phosphoglycolate generated by oxidative DNA damage and 3′-blocking lesions generated by alkylating agents, suggesting a broad role for TDP1 in the maintenance of genomic stability. TDP1-dependent repair pathways are normally redundant with other DNA damage response pathways that are often compromised in cancer cells. Moreover, checkpoint deficiencies are common in cancer cells, and in these cases TDP1 becomes the main mechanism for removal of Top1-mediated DNA damage. We hypothesize that combination chemotherapy with TDP1 inhibitors should synergize with topoisomerase-targeted drugs and enhance selectivity toward cancer cells with preexisting deficiencies in parallel repair pathways, giving rise to "synthetic lethality." Based on the growing number of TDP1 substrates, TDP1 inhibitors should not only synergize with Top1-targeting drugs (irinotecan, topotecan, and other camptothecin derivatives), but also with Top2 inhibitors (etoposide, doxorubicin, epirubicin) and gemcitabine, a drug used in the treatment of non-small-cell lung cancer. Currently, there are no validated inhibitors of TDP1 available in the clinic. The specific aim of this project is to discover novel inhibitors of TDP1 by crystallographic fragment screening and to optimize any leads that are obtained by structure-assisted drug design in order to propel these agents into clinical development. TDP1 lacking its N-terminal domain (residues 1–148), which is dispensable for enzymatic activity, was cloned, overproduced in *E. coli*, and purified. Crystals of truncated TDP1 (residues 149–608) typically diffract X-rays to a resolution of 1.6 Å at the APS synchrotron. The structure was solved by molecular replacement. The active site of TDP1 is exposed to solvent in the crystals, making them ideal for soaking with fragments. Using crystallographic fragment screening, we have successfully identified four small-molecule fragments that bind to the active site of TDP1. Using the high-resolution structures of Tdp1 in complex with these fragments, we are currently using structure-based drug design methods to optimize these fragments into more potent TDP1 inhibitors.

Discovery of an allosteric inhibitor of the E2 SUMO-conjugating enzyme Ubc9. The conjugation of the small ubiquitin-like modifier (SUMO) to protein substrates is an important post-translational modification that has ramifications for cancer and other diseases. As the sole E2 enzyme in the tightly regulated E1/E2/E3 SUMOylation enzymatic cascade, Ubc9 plays a central role in the conjugation of all three SUMO isoforms to many different protein substrates. Ubc9 is viewed as a promising yet challenging anti-cancer drug target. Indeed, past efforts have failed to yield any effective small-molecule inhibitors of Ubc9. Therefore, new experimentally validated chemical inhibitors of Ubc9 would provide insights into targeting of this important enzyme. Within the past decade, fragment-based drug design has emerged as a powerful approach to identify ligands for challenging protein targets that can provide excellent starting points for the development of potent inhibitors. By X-ray crystallographic fragment screening, we have identified two small-molecule fragments, biphenol and 5-chloro-2-mercaptobenzoxazole, that bind to Ubc9 at a pocket that is distal from its active site.

Binding of these fragments in solution was also confirmed using ^{1}H-^{15}N heteronuclear single quantum correlation (HSQC) NMR chemical-shift perturbation experiments. Although these fragments have weak affinity for Ubc9, biochemical and biophysical assays have confirmed that they inhibit SUMO conjugation. Site-directed mutagenesis of the binding-site residues E42A and K59A abolished inhibitory activity and confirmed that specific binding of the fragments to this site is responsible for inhibition. In order to further probe the structural basis for chemical inhibition, molecular dynamics (MD) simulations on Ubc9 in the presence and absence of fragments were examined by principle-component analysis, a technique used to reduce sets of multidimensional variables that describe conformational dynamics into nondegenerate components. The resulting MD data points toward a structural model whereby the fragment binding event modulates the dynamics of Ubc9 rather than its conformation and results in rigidification of the protein in general with pronounced effects near the active site loops in addition to the binding site. In summary, we have reported the discovery of a previously unknown allosteric binding site that could potentially be exploited for the development of novel allosteric inhibitors of Ubc9.

Bacterial Genetics Group

The Bacterial Genetics Group (BGG) uses the bacterial virus λ and its host, *Escherichia coli* (*E. coli*), as paradigms for ongoing developmental and gene regulation studies. Coevolution of λ with *E. coli* has produced genetic systems that are exquisitely connected to the most basic functions of the bacterial host. By examining the interface between λ and host systems, BGG follows the trail of the virus to understand what is most important and vital to cellular life and how all viruses might exploit cellular systems. The virus provides clues as to how those cellular functions work and how to study them. Recent characterizations of the λ genetic network have provided a framework for systems biology approaches using λ as a prototype for theoretical modeling methodologies, which have become important for addressing signal transduction, cancer development, and other complex genetic networks of eukaryotes.

BGG continues to develop recombineering, a highly efficient technology for precise in vivo manipulation of DNA in *E. coli* and other bacteria using genetic recombination. Recombineering at short (50 base pairs) homologies mediated by bacteriophage functions (collectively known as "Red") is used to modify mammalian genes resident on bacterial artificial chromosomes (BACs); these altered sequences can then be introduced back into the organism of interest. Recombineering with double-strand polymerase chain reaction (PCR) products is used to precisely replace a defined region with a drug marker, enabling the creation of null gene mutants. Recombineering with single-strand DNA oligonucleotides (oligos) 70 nucleotides in length is extremely efficient in the absence of methyl-directed mismatch repair, and 50–75 percent of the viable cells can become recombinant. Single-strand recombination enables point mutations to be created on large molecules, such as BACS, and these recombinants can be identified with a PCR screen. In bacterial genomes, this technology enables targeted mutagenesis and can be used to make specific mutations in even essential genes—the most interesting class of genes, yet often the most difficult to manipulate. BGG also studies a second recombineering system derived from a defective bacterial virus, the RecET system. Like the Red system, RecET consists of a single-strand annealing protein and a 5′ –> 3′ dsDNA exonuclease. Though poor for standard recombineering techniques described above, RecET is better than Red for fragment joining, which is an in vivo cloning method of assembling nonreplicating linear DNA fragments using short terminal homologies. BGG has found that the RecET system can efficiently assemble three different DNA fragments into a replicating plasmid when the individual DNA molecules are introduced into the bacterial cell by electroporation. They continue to characterize the parameters of this fragment-joining reaction, which may provide an alternative to Gibson assembly if a number of DNA pieces can be successfully assembled simultaneously in vivo. BGG finds that mutation of a bacterial 3' exonuclease stimulates recovery of recombinant products for fragment joining promoted by the Red system, and the group will work to identify other bacterial mutations that increase efficiency of this recombination. Thus far, BGG has found the λ Red system to be superior for all other recombineering reactions. They continue to characterize novel bacteriophage and prophage systems for their ability to promote recombineering reactions. The Phi80 prophage resident in the commonly used cloning strains DH5 and DH10B as well as the phage P22 from *Salmonella typhimurium* both have Red- and RecET-like systems that display robust activity in BGG recombineering assays.

The bacterial virus λ has two developmental life styles: active viral reproduction and lysogeny, or viral latency, where the virus exists as a prophage. It is of interest to understand how a quiescent virus is reactivated, since viruses are responsible for a number of human diseases, including cancer. For the λ prophage, the CI repressor protein prevents expression of most viral genes. BGG has devised a reporter system to monitor viral induction from the prophage state. This reporter system was used to demonstrate activation of the viral lytic promoters by a previously unknown mechanism. The *E. coli* cell maintains a proton (H^{+}) gradient across the bacterial cytoplasmic membrane. This gradient causes both a pH and charge differential across the membrane known as proton motive force (pmf) that is used to perform cellular work. BGG finds that when pmf is compromised with a class of drugs known as uncouplers, the λ CI repressor is inactivated, allowing the lytic promoters of phage λ to be activated. Like viral induction in response to DNA damage, this novel lytic promoter activity depends on the *E. coli* host RecA function. It was

not previously known that perturbation of pmf induces latent viral expression, and the work may have implications for pathogenic bacteria, since pathogenicity islands often contain prophages.

BGG has also identified two viral genes that are expressed in the λ prophage along with CI repressor that modulate viral induction, *rexA* and *rexB*. Genetic analysis shows that the RexA protein promotes induction of the virus, and the RexB protein controls this activity of RexA. In order to understand the molecular mechanism of these effects, BGG is working to identify host factors impacted by the Rex system and has engaged in a collaboration with a biochemist in the Department of Molecular Medicine at Cornell University, Dr. Josh Chappie, who has crystallized the RexA protein and is examining in vitro interactions of RexA, CI repressor, and the DNA operator sites to which CI binds.

BGG has developed a highly sensitive *cre/lox* gene reporter assay to analyze transcription misincorporation errors in *E. coli* cells. The novel assay allows BGG to specifically monitor transient transcription errors by preserving them as stable genetic changes, and it substantially minimizes the impact from translation errors by using a mutant Cre protein, the activity of which can be restored only at the level of transcription. The assay was optimized using G –> A misincorporation as a model. Utilizing recombineering technology and sampling various cellular growth conditions and assay media, BGG has developed robust and highly reproducible protocols for quantitative and qualitative analyses of transcription misincorporation errors in *E. coli*.

BGG has used the assay to study the role of transcription elongation factors GreA and GreB in transcription fidelity. Both GreA and GreB have been considered to be transcription fidelity factors because they have a similar activity of binding to RNA polymerase and induce its intrinsic RNA cleavage activity during transcription to eliminate misincorporated nucleotides in mRNAs. Single *greA*, *greB*, and the double *greAB* gene knockouts were made and tested in the newly developed assay. BGG found that the absence of GreA caused a 1000-fold increase in G –> A errors. The absence of GreB had no effect, either alone or in combination with GreA. These data indicate that GreA, but not GreB, is a transcription fidelity factor. Recently, mutations were generated within RNA polymerase at the binding site for GreA. These mutations altered two adjacent residues, and some combinations either reduced or increased nucleotide misincorporation by RNA polymerase. Thus, not only GreA mutants but also RNA polymerase mutants that affect GreA binding alter transcriptional fidelity.

Urologic Oncology Group

The focus of the BSP Urologic Oncology Group (UOG) within the Urologic Oncology Branch of CCR is the discovery and characterization of kidney cancer susceptibility genes through studies of families with rare inherited renal cancer syndromes and deep sequencing of sporadic histologically defined renal tumors. The goal of UOG is to provide insight into the molecular mechanisms that lead to the development of kidney cancer through functional studies of the proteins encoded by these kidney cancer susceptibility genes, and to apply this knowledge to the development of effective molecular targeted therapies. UOG has developed genetically engineered mouse models for in vivo studies of protein function and for testing novel therapeutic agents to treat kidney cancer patients.

Significant Achievements

The UOG manages over 200 patients and their family members with the inherited renal cancer disorder Birt-Hogg-Dube´(BHD) syndrome that is caused by germline mutations in the *folliculin (FLCN)* gene. Although *FLCN* sequence variants or intragenic deletions have been identified in greater than 90 percent of these BHD families, there remains a small group of families who have been clinically diagnosed with BHD for whom no *FLCN* alteration has been found. UOG has established a pipeline using large-insert whole-genome sequencing technologies developed by Pacific Biosciences to evaluate 40kb of genomic sequence encompassing the *FLCN* gene. They hypothesize that the remaining 10 percent of BHD families may have novel sequence variants in regulatory regions within introns or untranslated regions or a large structural rearrangement that would affect the level of FLCN expression. This approach has identified a *FLCN* coding sequence variant in the original BHD family reported in 1977 and used for genetic linkage to identify the disease locus that was previously not detected due to technical issues. Evaluation of additional BHD patients for noncoding sequence alterations in the *FLCN* genomic region is underway using this pipeline.

FLCN-deficient animal models are important research tools for evaluating therapeutic agents to treat BHD-associated renal cancer. Previously, UOG developed a kidney-targeted *Flcn*-knockout mouse model that developed highly cystic kidneys, leading to renal failure at three weeks of age. Biochemical analysis of the *Flcn*-deficient kidneys demonstrated mTOR activation, and rapamycin treatment was able to partially reverse the kidney phenotype. Based on these data, UOG evaluated the efficacy of rapamycin in a cohort of Nihon rats, a naturally occurring rodent model of BHD, which carries a *Flcn* germline mutation and develops renal tumors by six months of age. Magnetic resonance imaging (MRI) surveillance revealed stable disease in some of the rapamycin treated rats, although excessive weight loss led to early termination of the study. Plans to test a dual mTORC1/mTORC2 inhibitor and/or rapamycin in combination with a second agent are underway. As a result of promising in vitro drug screens performed in collaboration with the National Center for Advancing Translational Sciences (NCATS) using a *FLCN*-deficient human renal tumor cell line, efficacy studies of several other agents that target the proteasome, microtubules, or angiogenesis were conducted in the Nihon rat model, but

these drugs had no effect on *Flcn*-deficient renal tumor growth. Studies in the *Flcn*-deficient rodent models evaluating additional therapeutic agents identified through the NCATS drug screen are in progress.

The transcription factor *TFE3* located on chromosome Xp11.2 has been identified in fusion with other genes in sporadic renal cancers, known collectively as TFE3-translocation renal cancer. The gene fusion renal cancers are rare in adults but comprise 20–50 percent of all childhood renal cancers. Xp11.2 translocation renal cancer is highly aggressive with poor prognosis, and no effective treatment is currently available. UOG has generated a *PRCC-TFE3* knockin mouse in which a *loxP-STOP-loxP-PRCC-TFE3* cassette was inserted into the *ROSA26* locus, and the STOP codon was deleted genetically by crossing with *CDH16 (cadherin 16)-Cre* mice, resulting in *PRCC-TFE3* fusion gene expression specifically in the kidney. These mice develop multiple cysts and tumors, which are evaluable by MRI after six months of age, thereby providing a valuable research tool for elucidating molecular mechanisms of TFE3-fusion renal tumorigenesis and for therapeutic drug testing. Based on promising results from NCATS drug screens in cell lines established from human TFE3-fusion renal tumors, UOG evaluated several drugs in this in vivo model that are classified as inhibitors of the proteasome, BCR-ABL, or angiogenesis, but were unable to detect a tumor response with these agents by MRI. Continued drug screens with NCATS in the human TFE3-fusion cell lines are ongoing, which will inform future drug studies in the TFE3-fusion mouse model.

Flow Cytometry Group

The CCR-Frederick Flow Cytometry Core (the Core) is a dedicated resource embedded in the CIP for CCR investigators at NCI at Frederick. Its services include analyzing and/or sorting stained or transfected samples, and training individuals to understand the principles of flow cytometry so they may ultimately operate the analyzer instruments that the Core makes available to them. These services enable investigators to use flow cytometry to design experiments that will yield reliable data. The staff also consults with investigators to develop and refine new flow cytometry techniques. To serve the needs of CCR investigators at NCI at Frederick, the Core houses five analyzers and three cell sorters. The instruments vary in capability, ranging from having two lasers with the ability to detect four fluorochromes to having three to five lasers capable of detecting up to 18 fluorochromes or antibodies. There are two sorters and two analyzers with the same capabilities so that there is more flexibility in the instrumentation for the investigators to find an instrument that meets their requirements for analysis and sorters that can be used to sort their populations of interest without having to modify their configurations.

SIGNIFICANT ACHIEVEMENTS

The Core is an indispensable resource for NCI at Frederick investigators. In FY2016, 66 investigators from 36 laboratories have used the expertise of the Core staff and the instrumentation maintained by the staff. During the past year, the Core has encouraged training of investigators so that they may run their own analysis experiments, allowing the staff more time to concentrate on cell sorting. A new and more user-friendly online instrument scheduling calendar was designed and implemented this year with the help of BSP and Frederick IT staff. The Core staff and instrumentation have provided cell-sorting support for limb bud and allantoic cell differentiation of transgenic mice expressing tdTomato and GFP fluorescent proteins, characterization of human myeoloid-derived suppressor cells, developing cell lines expressing high levels of protein expression for further analysis, and creating libraries of yeast expressing transfected genes. The flow core also provided support for the following studies: differentiation of mesenchymal stem cell, studies of chronic expression of gamma interferon, developing a panel to monitor possible anti-cancer therapeutic compounds, a BRACA2 minor transcript that possibly accounts for survival in human breast cancers, and cell cycle progression regulation through a proteasome ubiquitin receptor. During the first three quarters of the year, the Core staff trained 17 investigators, including 5 from CCR labs outside of the CIP, to run their own samples, thus greatly increasing the output of the flow instruments. Without individual investigators acquiring and analyzing their own data, the government would need to hire two or more full-time, experienced individuals to perform the same volume of work. Between October 2015 and July 2016, the Core staff sorted 303 samples and ran 5,880 samples for various investigators. During the same period, at least 18 papers have been published by investigators using data generated using the Core instruments.

Media Laboratory

The Media Laboratory has been in operation since 1984, and its employees are skilled at making microbiological media for both bacterial and yeast work. They routinely make buffers and other reagents for biochemistry, molecular biology, and genetics research. All media are custom-made (liquid and plates); thus, additives such as antibiotics, isopropyl β-D-1-thiogalactopyranoside (IPTG), counter-selective agents, anticancer drugs, and reverse transcriptase inhibitors, can be added at the request of the researcher. (Ingredients not commonly used by most labs need to be provided by the ordering lab.) The Media Laboratory is usually able to accommodate requests for new reagents when provided with a recipe. Because it is located on the NCI at Frederick campus, the laboratory's staff members are available to answer any questions. They can also accommodate requests for dispensing products in a variety of sizes (e.g., 10 bottles of LB at 100 ml/bottle).

All products use the highest-quality reagents: only Difco agar, Tryptone, and yeast extract are used. Most products are delivered within three days of the order. At present, the laboratory provides services to about 50 laboratories. On-site media and reagent preparation is a highly cost-effective and valuable resource for many scientific laboratories that require microbiological media or other molecular biology reagents.

Recently, a more reasonable cost-sharing pricing system was implemented to replace the previous flat-rate system. Under the cost-sharing system, buffers, plates, and reagents fall into different pricing tiers, depending on the cost of reagents and labor.

Media Laboratory Products

- Bacterial growth media: LB, TB, and minimal broth and plates using Difco products, with or without antibiotics or other additives
- Diagnostic growth media: MacConkey agar with sugar of choice, EMB agar with sugar of choice
- Yeast growth media: YEPD, YEA, drop-out plates
- Buffer and solutions: Tris buffers, Q-buffers
- Gel electrophoresis and transfer buffers: Tris-glycine buffer, SDS-PAGE, TAE
- Bacteriophage growth media for phage lambda and M13: TB, NZY, YT.

The Media Laboratory provides services to NCI CCR laboratories at two Frederick locations, the NCI Campus at Frederick and the Advanced Technology Research Facility, as well as to the laboratories at NIH Bethesda.

Basic Science Program CCR Genetics Core

The BSP CCR Genetics Core (BCGC) is involved in the genetic research of CCR investigators. The BCGC provides support to CCR investigators in three main areas—bioresources, genotyping, and bioinformatics. The BCGC works closely with investigators in the initial stages of cohort development, sample processing, storage, support, and maintenance of patient samples and data associated with each patient. BCGC has been tasked with continued support to a core group of principal investigators—Carrington, Dean (discontinued mid-year as Dean lab is now at DCEG), McVicar, and Winkler. In total, BCGC has supported many researchers in CCR and its programs, including the CCR OD and BSP administration.

The BCGC has been very productive scientifically this year, with nine articles published and 21 submitted that utilized the BCGC services. BCGC researchers remain an integral part of the core PIs research from the start to finish in their studies. The BCGC consisted of six personnel for the beginning of the fiscal year, but with the retirement of one person, the Core is now a five-person group that will be downsizing again before fiscal year ends. The current personnel continue to support and aid researchers with their research projects and sample support.

Cohorts

This section involves no personnel; the monies are provided to develop new cohorts of patients for study by CCR investigators. Internal grants, approved by CCR, are provided to investigators and are used to collect new clinical data, process tissues for research, and archive new samples for future studies. This fiscal year, our lab saw the addition of 12 new cohorts and expansion within 10 existing cohorts.

Bioresources and Genotyping

The bioresource and genotyping sections process samples received by CCR investigators (e.g., permits, cell culture, tissue extraction, entering into database systems, genotyping, etc.). Shipping permits, IRB approval, OHSRP, data transfer agreements, and material transfer agreements (MTAs) are managed by BCGC staff and tracked in the BCGC database. To ensure that all patient data and samples collected are in accordance with NIH policies, all entries in our database system are able to be cross-referenced with their pertinent paperwork. In the fiscal year 2015, the BCGC processed four Institutional Review Board (IRB) reviews, 55 Office of Human Subjects Research Protection (OHSRP) approvals, 22 request for patients from the Research Donor Program Requests (RDP), two Multicenter AIDS Cohort Study (MACS) Concept Sheets, and 17 NIH Quarantine Permits. There were also numerous export and import permits, 14 MTAs, simple letter agreements (SLAs), and data agreements obtained for use by our CCR investigators. All clinical data and samples received by the BCGC are barcoded and entered into the database system, which allows all "study pertinent information" to be available to CCR researchers and their collaborators (with proper security credentials) at any time during processing, data collection, and analysis of these samples.

A major success of the BCGC in its sample management and handling is the reliance on bar-coding, robotics, and database integration. Samples are received, bar-coded, and scanned into our database system during each phase of processing. Each sample is associated with all relevant information collected on that specimen. All core PIs use the BCGC's processing system in all samples that are handled by BCGC and in their own labs. This has greatly facilitated research in the CCR labs that utilize our system by reducing errors and increasing efficiency. This type of tracking has made the clean sweep required by NIH a much easier task. Our system tracks over 9 million DNAs on plates and tubes and close to 1 million tissue samples. In total, over 10 million samples are archived in our database system from over 201 cohorts. In the last year, the BCGC has handled over 4,338,932 ngs of DNA and 97,064 samples.

Investigator or BCGC samples are fed into the BCGC genotyping section, where state-of-the-art genotyping and sequencing is used to provide genetic data to investigators. The genotyping staff is trained on a number of low- and high-throughput systems. During this year, they have generated 100s of millions of genotypes on various high-throughput genotyping platforms for our PIs. The BCGC

has sequenced 84,384 samples on their ABI3730 and handled over 195,199 samples on their robotics, all targeted at sequencing. This past year has also seen the addition of the iSCAN system from Illumina to the BCGC. We have the capability of running the Infinium and Methylation systems by Illumina. The BCGC has also completed the necessary training to work on the MiSeq Platform and are currently ready to run this platform once a project is submitted. We continue to expand our expertise to better serve our clients.

In addition to the sample handling and genotyping that the BCGC does, Bioresource and Genotyping personnel provide constant assistance to investigators in the CCR. Core staff facilitate research in CCR investigators' labs by refining and improving protocols, training other labs on techniques (especially with students and visiting scientific staff), providing space and equipment that other investigators don't have in their own labs for projects and studies, and extra hands on studies when needed. All this is accomplished by the BCGC staff because they are available locally, highly skilled, cross-trained, and interested in the science that goes on at the CCR.

Traditional, New, and Emerging Technologies Supported

High-Throughput Genotyping We have continued our extensive involvement in array-based genotyping, refining the SNP calls for the GWAS and AIDS, HBV, and kidney disease. We have utilized the iSCAN Illumina system this budget year. We are constantly exploring new high-throughput technology to enhance data collection on our samples.

Mi-seq Illumina System. The BCGC has received training in this new platform in the hopes of providing our CCR investigators with targeted gene sequencing, metagenomics, small-genome sequencing, targeted gene expression, amplicon sequencing, and HLA typing.

Cell Culture. In addition to normal immortalization of cells for current and future experiments, our cell culture group has also been maintaining HEK293 cells for weekly and biweekly experiments in which the cells are transfected with proper gene and control constructs. These have been used for FRET/FLIM exploration by one of our collaborators. Our cell culture group has also expanded cell lines in order to get needed quantities for RNA expression assays. We also have a collaboration with USAMRIID involving stable APOL1 cell lines for infectious disease testing and a project involving CrispR project in Saleem-LY podocyte cell line. Finally, the group has undertaken a mouse cell line project to establish cell lines from specially bred mice (neo/neo) and controls, make stock freezes, and test G418 tolerance levels.

Cancer and Inflammation Program Genetic and Microbiome Core

The Microbiome and Genetics Core (MGC) of the CIP has established its microbiome facility in Building 37 on the Bethesda campus over the past year, entailing considerable turnover in personnel and equipment to meet the growing interest and challenges of characterizing the role of the microbiota in cancer and inflammatory processes. The team now assembled consists of a research technician, two bioinformaticians, and two scientists, one who recently joined at the end of July 2016. The scientific challenges that were met over the past year range from experimental (establishing reliable and reproducible methods to isolate and characterize nucleic acids of microbiota isolated from feces and other sources) to bioinformatic (construction of bioinformatics pipelines to effectively determine changes in microbial representation between experimental samples).

Robotic sample preparation platforms (Eppendorf 5073 and 5075) were harnessed to maximize throughput and reproducibility, both for nucleic acid isolation and for barcoded library preparation. Quantification is accomplished using qPCR or Follow purification, barcoding and quantification, and an Illumina MiSeq system is used to sequence amplicons of 16S rRNA genes. Among the MGC's more important challenges is ensuring high reproducibility of data and analyses and to offer considerable comprehension of the bioinformatics output while being able to maintain a very low cost to users. To date, samples from more than 20 projects have been processed from inside CIP and NCI as well as with outside collaborators, and approximately 200 Giga base pairs of sequence data has been generated and analyzed from the MiSeq platform. Across the projects, challenges ranging from how to isolate DNA from high- or low-bacterial biomass sources, how to partition analyses from different sources, and which treatments will maximize the signal-to-noise ratio of experiments have been met successfully.

The bioinformatic challenges began with storage, delivery, and backup of large amounts of information. This was achieved using both Illumina's cloud server as well as a backup system at the computer center of FNLCR. Two analytical approaches to determining microbial abundances were utilized, and the Qiime and mothur platforms have been tested extensively. Over the past year, we have compared both and maximized their suitability for our purposes through modifying our analysis pipelines to take advantage of components of each. The analyses are also limited by the quality of databases of ribomsomal RNA. We developed a database of fully vetted, high-quality rRNA sequences for use in identifying components of the microbiome in samples.

To further explore the microbiome and move to metagenomics, we recently acquired the higher-throughput Nexseq platform from Illumina. We have already generated almost 100 Gbp of data on this machine and explored the bioinformatics challenges in going from defined amplicon targets such as rRNA to whole-genome or transcriptome sequencing. We are exploring software (Picrust, Pathoscope) that offers insights into the genomic data generated.

We continue to support analysis in genetics of HLA expression. Over the past year, we have been involved in the production of papers determining the characteristics of

promoter regions of HLA-A, -B and -C in relation to expression of these genes. We have been instrumental in helping to show that expression affects outcomes of infectious and autoimmune disease, such as HIV, as well as infection and transplantation of Crohn's disease. The genetic elements that control expression are of considerable interest, and we continue to support groups working on their characterization.

Support Provided by the Cancer Research Technology Program

Sequencing Facility

The primary mission of the Sequencing Facility (SF) is to utilize high-throughput sequencing technologies to enrich cancer research and ensure that the NCI community can remain on the leading edge of next-generation sequencing technology. The Center for Cancer Research (CCR)-funded SF provides CCR and NCI investigators with access to one MiSeq sequencer, three NextSeq 500 sequencers, and two Illumina HiSeq 2500 sequencers, one state-of-the-art HiSeq 3000, and Pacific Biosicences RS II sequencer. In addition, a Chromium 10X Genomic Platform for Single Cell and Structural Variation was brought into limited production at the SF this fiscal year.

SIGNIFICANT ACHIEVEMENTS

- The SF operated as a fully functional core facility in support of 75 investigators largely from CCR, but also from the Division of Cancer Epidemiology and Genetics (DCEG) and the Division of Cancer Treatment and Diagnosis (DCTD), along with the National Human Genome Research Institute (NHGRI), National Institute of Allergy and Infectious Diseases (NIAID), and Frederick National Laboratory for Cancer Research (FNLCR).
- As of July 2016, the Illumina Sequencing Laboratory had processed and sequenced more than 3,597 samples for 75 CCR principal investigators, and delivered more than 30 trillion bases. The SF Illumina Sequencing Laboratory also generated more than $887,000 through Core Services Accession System (CSAS) chargebacks.
- Several new sequencing and data analysis pipelines were developed by the SF team, including structural variation detection, DNA Methylation, Single-Cell RNA-Seq, RNA-seq for FFPE, and low input RNA.
- As of July 2016, the Pacific Biosciences (PacBio) Sequencing laboratory has processed and sequenced more than 224 samples for 21 principal investigators.
- The PacBio laboratory has successfully applied the low input protocol for sequencing and assembly of Oat Crown Rust genomes. The team has also successfully completed a number of H. Pylori genomes, which has led to a much larger project, a collaboration between CCR and DCEG.
- During the FY2016, the CCR-SF Bioinformatics Group has provided sequencing analysis support to more than 80 laboratories from various branches across the CCR (including 75 laboratories that used llumina sequencing and 12 laboratories that used pacBio sequencing).
- In addition to production support, the CCR-SF Informatics group has worked with SF lab scientists actively engaged in technical development to expand the current next-generation sequencing (NGS) pipelines. Several new data analysis pipelines have been developed, including structural variation detection, PacBio targeted Iso-seq transcriptome analysis, DNA methyl-seq analysis for detecting base modification, RNA-seq analysis for low-input and low-quality formalin-fixed, paraffin-embedded (FFPE) samples, and single-cell RNA-seq analysis.

CCR-Dedicated Core Service Programs

CCR Protein Expression Laboratory

The 5.25 full-time equivalent (FTE) employees in the CCR-dedicated Protein Expression Laboratory (PEL) carry out cloning, virus production, protein expression, and protein purification in support of CCR activities. In order to better serve CCR, this group was expanded this year by one FTE to accommodate the large-scale insect cell protein expression needs of the structural biology groups within the Laboratory of Cell Biology (LCB) of CCR.

- The Cloning and Nucleic Acids Group (CNG), led by Carissa Grose, constructed 194 entry and expression clones for 40 investigators (68 CSAS requests by the end of July 2016) in FY2016. Nearly half of these clones were sent back to investigators for in vivo work in model organisms and cell lines. A quarter of the constructs were used for lentivirus production, and the remainder were used for protein expression work in the Protein Expression Laboratory (PEL). Almost all of the cloning and subcloning was done using Gateway recombinational cloning on our in-house-developed combinatorial cloning platform (CCP). The Virus Production section of CNG worked on 39 CSAS projects in FY2016 for 22 investigators and produced 75 lentiviral supernatants, most of which were titered by CNG prior to delivery.
- The Protein Production Group (PPG), led by Jane Jones, provides protein purification and production of cells and cell-derived products via expression in bacterial, insect, and mammalian systems. Primary expression activities include production of cytoplasmic and secreted recombinant proteins from *E. coli*, baculovirus-infected insect cells, and transfected mammalian cells. Following expression, PPG carries out high-throughput, micro-scale purification methodologies to screen samples for

positive lead constructs and/or expression conditions, and then proceeds to scale-up purification using affinity, ion exchange, and size-exclusion chromatography to purify native and recombinant proteins. During the time period covered, PPG worked on 45 projects for 32 investigators. Approximately 60 percent of this work focused on insect expression work in support of investigators in the CCR LCB. This group had high demands for expression work in support of structural biology studies, which led to the addition of a staff member to help support this work and still permit us to work on other CCR protein production projects. The support for LCB led to the production of more than 140 liters of insect cell culture as of July 2015. PPG carried out more than 20 protein purification scouting projects for 12 investigators and performed scale-up purification for a total of 21 projects. PPG work for CCR outside of the LCB projects generated over 60 baculoviruses in FY2015 and produced over 150 liters of expression materials.

CCR Protein Characterization Laboratory

The CCR-dedicated Protein Mass Spectrometry and Characterization Unit was funded through a Yellow Task (YT) with six FTEs. It provides crucial proteomics and mass spectrometry services for the NCI/NIH research community, ranging from macromolecular interactions, biochemical analysis of proteins, protein intact mass, identification, quantification, post-translational modification site mapping, and lipid and metabolite quantification.

SIGNIFICANT ACHIEVEMENTS

- In the past year, the unit has been working with 50 principal investigators on many projects. As a result, the unit has co-authored 12 publications in scientific journals, such as *Mol Cell* and *Cell Rep*.
- This year, the unit has successfully installed two new mass spectrometers for proteomics and small molecule analysis. The instruments are fully tested and functional and have joined the service fleet. These new additions will increase our capacity and decrease the turnover time.
- The unit has used the newer generation of high-performance liquid chromatography (HPLC) and high-resolution mass spectrometers to push the detection limit further for endogenous protein complex analysis.
- The metabolite and lipid analysis development has taken off in the past year. We have developed the quantitative assays for a panel of metabolites in the Krebs/time course assay (TCA) cycle. We have had significant increases on the number of requests, as well as the complexity of the requests.
- During the last six months, PCL has had a significant increase in surface plasmon resonance spectroscopy (SPR) requests from NCI. The laboratory has been able to respond to this demand due to its flexible research staff.

CCR Optical Microscopy and Analysis Laboratory

The Optical Microscopy and Analysis Laboratory (OMAL) focuses research on quantitative microscopy for understanding carcinogenesis by analyzing signaling pathway kinetics and molecular spatial organization in individual cells. Research is in collaboration with multiple CCR principal investigators. Technical developments aim to provide an integrated resource for analysis of biological samples across multiple scales, from the molecular to the animal level, thus providing a seamless, bi-directional transition between basic and translational research.

This report covers the duration of August 7, 2015 through August 1, 2016.

OMAL continues to provide substantial microscopy and image analysis support to NCI, with the level of usage of the microscopes increased over last year. The number of users increased by 7% and the number of new users increased by 27%. OMAL trains microscope users to be independent in order to maximize productivity. OMAL recognizes that its aging equipment is no longer cutting-edge, which is a significant drawback to NCI research. Therefore, significant effort was undertaken to procure new fluorescence microscopy instrumentation. The multi-functional microscope (MFM) was finalized, construction of a second MFM for HIV research commenced, a laser scanning confocal microscope was upgraded for the detection of near infra-red emitting dyes, and stochastic optical reconstruction microscopy (STORM) was added to the structured illumination microscope. Extensive evaluation of different light sheet microscopes was undertaken, resulting in a combination of a laser scanning confocal, spinning disk confocal, and light sheet microscope being ordered. Instrumentation at the Advanced Technology Research Facility (ATRF) is available for CCR use when not needed by the RAS program.

Thermo Scientific is loaning a Raman confocal microscope to OMAL, which is currently being evaluated to detect endogeneous chemical changes in fixed cancer and non-cancer cells and tissues. Forthcoming work will evaluate surface-enhanced Raman spectroscopy in live cells. CCR collaborators are Dr. Mioara Larion, Dr. Karlyne Reilly, and Dr. David Wink.

A major new effort during this year was the award to Laboratory-Directed Exploratory Research funding by Leidos Biomed to model cell heterogeneity dynamics in tumors in response to drugs. The project is in collaboration with Dr. Robert Kinders (Head, Pharmacodynamics Section, Laboratory of Human Toxicology and Pharmacology, NCI at Frederick) and Professor Konstantina Trivisa (University of Maryland, College Park). A key accomplishment was to show that tissue cleared with thiodiethanol could be imaged at high 3-D

spatial resolution with two photon microscopy to a depth of 190 µm. Furthermore, automatic segmentation software could detect the isolated cell nuclei, while clustered cell nuclei could be segmented by novel interactive software developed by us (Figure 1).

SIGNIFICANT ACHIEVEMENTS

- Cutting-edge methods to segment cells and cell nuclei from 3-D images using graphcut methods were reimplemented in "Python" to facilitate free distribution to users.
- Added laser micro-surgery to a confocal microscope in the laboratory of Dr. Jadranka Loncarek.
- Built software to evaluate the proximity of gene loci to each other and to the nuclear envelope in support of research by Dr. Petr Kalab (CCR – Bethesda).
- Continued enhancement of the website, including microscope training material.
- Following demonstration of the ability to perform single-molecule fluorescence resonance energy transfer (FRET) when molecules are attached to the substrate, further studies with a Cy3-Cy5 labeled DNA sample showed evidence of two states: one exhibiting FRET and the other not exhibiting FRET. In support of Dr. Yun-Xing Wang (CCR – Frederick).

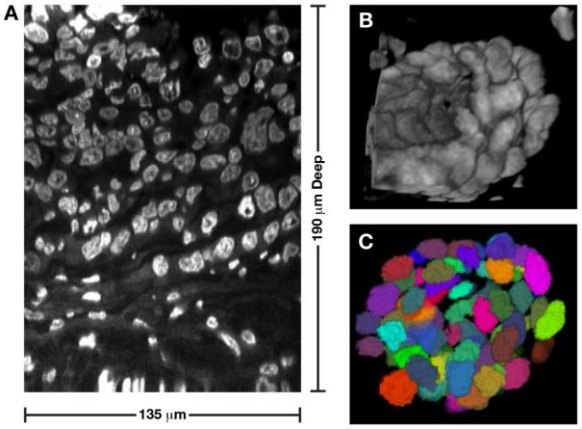


Figure 1: A) Depth slice of a sample of the human gastric cancer cell line, MKN45 grown as a xenograft in the mouse, labeled with the DNA dye, DAPI, and imaged to 190 µm depth with two photon microscopy. B) 3-D image of cell nuclei in an epithelial cell structure in mouse skin. C) Identification of the individual cell nuclei using in-house segmentation software.

Examples of collaborations OMAL conducted with CCR labs:

- 3-D live cell imaging showed that IFNγ limited directed movement of metastatic melanoma cells. Abstract submitted. Further studies investigated macrophage and melanoma cell interactions. With Dr. Julio Valencia and Dr. Howard Young, CCR – Frederick
- Study of the dimerization of APO-L1 protein and mutations of the protein in acidic compartments of cells using FLIM – FRET. Fluorescence anisotropy was also tested, but was not successful. With Dr. Cheryl Winkler, CCR – Frederick.
- 3-D super-resolution imaging of the distal appendage protein cep164 surrounding the centriole imaged with STORM and compared to SIM imaging. A post-baccalaureate, Matt Bowler, was recruited to develop new protocols and to test new STORM labels. With Dr. Jadranka Loncarek and Dr. Martin Schnermann, CCR – Frederick.
- Quantified increase in stress fibers in cEBPD KO cells cultured on glass and on micropatterns, and the reversal of the effect by alpha-catenin. With Dr. Esta Sterneck, CCR – Frederick.
- Study of the mobility of the HIV matrix domain in supported lipid bilayers. Study utilized total internal reflection flourescence (TIRF) microscopy, a unique reaction vessel, and development of stringent cleaning protocols for coverslips. With Dr. Alan Rein, CCR – Frederick.
- Investigating interactions of Mad2 protein in live cells by single molecule imaging. Halo and SNAP Piggybac constructs were made and imaging experiments confirmed the enzymes are in the right location. With Dr. Steven Hou, CCR – Frederick.
- FCS measurements were performed to monitor MAX1 peptide size and dynamic changes in different buffer conditions and as a function of time. With Dr. Joel Schneider, CCR – Frederick.
- Live cells tagged with smoothened-RFP and Rab-GFP were imaged with the MFM. With Dr. Chris Westlake, CCR – Frederick.
- Quantification of polymerase ligation assay signals in cells to determine RAS protein dimerization. With Dr. Nadya Tarasova, CCR – Frederick.
- Fluorescence fluctuation techniques to investigate HIV particle assembly in single live cells. The diffusion dynamics of Gag-mCherry, mutated Gag-mCherry, and mCherry alone were successfully measured using fluorescence correlation spectroscopy. mCherry and Gag-mCherry were also analyzed using raster image correlation spectroscopy. With Dr. Wei-Shau Hu, CCR – Frederick.
- Developed software to measure mRNA foci and DNA distribution in bacteria. With Dr. Ding Jin, CCR-Frederick.
- Quantitative evaluation of FCS and FRET to support biophysical studies, including fluorescence correlation spectroscopy analysis of HIV Gag and RNA interactions and DNA interactions. Introduced "Alternate excitation" FRET as an improved method to detect FRET and to measure stoichiometry. With Dr. Rein (CCR – Frederick) and Dr. Rajat Varma (formerly NIAID).

- STORM imaging of the bcr protein in B cells. With Dr. Young (CCR) and Ven Natarajan (NIAID).
- Investigations of the effects of nitric oxide (NO) on cell morphology in conjunction with reversal of epithelial to mesenchymal transition. Initial experiments quantified the half-life of DAF fluorescence, a marker of NO in cells. CCR collaborator: Dr. Wink.
- Developed software to measure the localization of CEPB and PPiB RNA in polarized cells. In collaboration with Dr. Peter Johnson.
- Developing software to localize Rab13 RNA in 3-D spheroids. In collaboration with Dr. Voula Mili.

Electron Microscopy Laboratory

The Electron Microscopy Laboratory (EML) supported CCR with at least two FTEs during FY2016. EML has supported several challenging projects from NCI CCR (e.g., cryo-electron microscopy (EM) of mutant murine leukemia virus (MLV) for Dr. Rein's group, cryo-EM and Scanning Electron Microscopy (SEM) of lipogels for Dr. Schneider's group, imaging of fly brain for Dr. Jairaj Acharya's group, imaging of spherical protein complexes for Dr. Kyung Lee's group). An FEI T12 microscope for a Fort Detrick location has been purchased, a suitable temporary location has been identified, and the room has been renovated. A Scientist I has been hired to support and help users at the Fort Detrick location with the use of the instrument.

Center for Molecular Microscopy

The Center for Molecular Microscopy (CMM) is a state-of-the-art lab supported by 5.5 FTE with cutting-edge microscopes for 3-D imaging of cells and tissue using FIB-SEM technology and to perform high resolution cryo-electron microscopy. CMM is running more than 20 active collaborations with the CCR and the RAS program.

Significant Achievements

- During the last year, the FEI Titan Krios for high resolution cryo EM was installed, tested, and has been signed off. The complete workflow at the ATRF, including the instrument, has since then been used for acquiring several datasets, some of which resulted in reconstructions at high resolution (around 4–5 Angstrom) verifying that the instrument is performing at full capability.
- The Zeiss Crossbeam 540 instrument for FIB-SEM imaging of cells has been installed and signed off. This instrument has been used already to produce numerous datasets for CCR collaborations. For example, on cilia development with Dr. Westlake's group, the role of TIM1 in HIV budding with Dr. Eric Freed's group, T-cell activation with Larry Samelson's group, and chemotaxis in dictyostelium with Dr. Carole Parent's group.
- A high-pressure freezer (Leica ICE) was purchased, tested, and signed off. This instrument is used to develop high-pressure freezing protocols and workflows to supplement and replace existing room-temperature embedding protocols.

Genomics Laboratory

The CCR-dedicated Genomics Laboratory (GL) is funded by a YT through CCR with five FTEs. The primary mission is to provide dedicated genomics services to CCR laboratories. The GL provides a broad range of genomics services, including gene expression analysis such as microarray, qPCR, droplet digital PCR, NanoString, and Fluidigm services; mutation analysis; single-nucleotide polymorphism and copy number analysis; DMET array; and next-generation sequencing services, including 16s microbiome analysis, integration site analysis, and exome sequencing in conjunction with the CCR Sequencing Facility.

Significant Achievements

- During last year, the GL worked with over 60 CCR investigators and fulfilled 141 CSAS requests and over 3,500 total samples. We processed over 3,500 samples, including 449 for Exome sequencing, 919 for microarray, 765 for NanoString, and 786 for MiSeq 16s metagenomis or amplicon.
- The GL sees increased demand for exome-sequencing projects. We processed 21 CSAS requests and 452 samples. The work order is worth $360,160, a 50% increase from the previous year.
- The GL continues to see strong demand for microarray service. We processed 54 CSAS requests and 919 samples.
- The GL upgraded our NanoString platform to a GenII scanner with additional CCR funding. Our throughput is not improved. We saw strong demand for expression analysis on targeted gene panel. We processed 18 CSAS requests and 765 samples.
- The GL introduced a few new genomics services for CCR investigators. These include ImmunoSeq, HTG EdgeSeq, Affymetrix OncoScan, and DropSeq single-cell sequencing. The ImmunoSeq is a technology to assay clonality of T cells based on T-cell receptor sequence diversity. It provided a very useful tool for NCI investigators for cancer immunotherapy studies. The HTG EdgeSeq provided a quantitative method for gene expression using FFPE samples without sample extraction. The new Affymetrix OncoScan array provided a cutting-edge technology for CNV analysis in FFPE tumor samples. We already process more than 200 tumor samples on OncoScan arrays.
- The GL also provided in-house developed high-throughput integration site analysis for many NCI investigators. This technology has a great impact on many of the research projects at NCI, such as

Dr. Steve Hughes/Dr. Frank Maldarelli's HIV clonal expansion study, Dr. Scott Durum's T-cell leukemia study, Dr. Dan Larson's gene-trap gene expression imaging and nuclear structure study, Dr. Phil Tofilon's gliablastoma PDX tumor clonality study, and Dr. Dennis Hickstein's lentivector gene therapy study.

Support Provided by the Laboratory Animal Sciences Program

Molecular Imaging Laboratory

The Molecular Imaging Laboratory (MIL) develops new methods for preclinical and clinical in vivo imaging in support of the NCI Molecular Imaging Program (MIP) in Bethesda. MIL supports a collaborative effort between MIP, the Urology Oncology Branch (UOB), and the NIH Center for Interventional Oncology on focal therapy of prostate cancer by developing diagnostic magnetic resonance (MR) imaging and analysis methods to localize and monitor the lesions, and to guide interventional devices for targeted diagnosis and therapy. MIL is directly involved in a new NCI director initiative in collaboration between MIP, the Radiation Biology Branch, and UOB to study cancer metabolism by mapping injected metabolites and their conversion to other metabolites in patients, using hyperpolarized ^{13}C MRI.

The preclinical component continues to focus on the development and application of novel methods and instrumentation for small-animal optical, MR, and radionuclide imaging to complement MIP's effort in developing new diagnostic and therapeutic agents. In addition, the group provides imaging expertise to the SAIP at the FNLCR and the NCL at the Advanced Technology Research Facility (ATRF).

Significant Achievements

Work on the NCI major opportunity project to study cancer metabolism using hyperpolarized ^{13}C MRI continued. The first dual-probe in vivo hyperpolarized ^{13}C-pyruvate and ^{13}C-succinate study on mice was performed utilizing a mouse leg coil on the clinical Philips MRI 3.0 T scanner and utilizing the Spinlab polarizer. MIL designed, tested, and successfully implemented a new coil ($^{15}N/^{1}H$) for the Philips 3.0T scanner to perform novel hyperpolarized studies to take advantage of the longer T_1 relaxation time compared to ^{13}C. MIL also designed and built specialized quadrature ^{13}C/linear ^{1}H mouse coils (torso and head) with associated animal support systems (heating and anesthesia) to perform mouse studies on the cryogen-free 3.0 T small animal MRI (MR Solutions) that operates with the preclinical hyperpolarizer (Oxford Instruments HyperSense) in the Radiation Biology Branch (RBB). These coils can also be interfaced to the Philips 3.0T clinical scanner.

MIL was also instrumental in numerous projects that include: 1) developing coil interfaces for both clinical and preclinical studies which the vendor was unable to provide and resulted in improved images (higher signal-to-noise); 2) designed, built, and implemented a hand-held IR fluorescence scanner for the operating room for the detection of a therapeutic IR 700 dye-antibody conjugate for targeted photoimmunotherapy; 3) designed and built enhanced 3D-printed dual mouse imaging tables for PET and SPECT scanners for both MIP and SAIP facilities, including animal support systems (heating and anesthesia); and 4) improved the process for rapid generation of patient-specific 3D prostate molds for cancer therapy using the patient specific MRI scan. MIL continued to support multiple preclinical PET drug development projects with novel imaging systems constructed in-house and analytic methods intended to improve the efficiency and accuracy of these studies.

MIL personnel, along with their collaborators, published three journal articles, conducted at least three presentations, and developed several posters for international scientific meetings during the past year.

Center for Advanced Preclinical Research

The mission of CAPR is to develop strategies for predictive preclinical research using genetically and biologically engineered murine cancer models, and to facilitate their routine application in clinical research to achieve optimal outcomes in the management of cancer diseases. Early genomic, biomarker, and preclinical drug assessment studies of recent years have illustrated both the value of using well designed GEM models to accelerate biomarker/molecular signature discovery, and the potential for significantly increased accuracy in efficacy determination. CAPR is dedicated to developing preclinical approaches that can be integrated into the routine practice of human research to improve overall clinical care, including the process of selecting novel treatment strategies for clinical trials.

Significant Achievements

At present, responding to CAPR's evolving mission of advancing the program's preclinical capabilities to support intramural translational research and guided by the recently formed Center for Cancer Research (CCR) Scientific Oversight Committee, CAPR is involved in facilitating the submission of collaborative proposals prepared by CCR investigators. A request for applications prepared by CAPR in consultation with the oversight committee was distributed to CCR PIs soliciting submissions with the purpose of supporting rigorous preclinical studies in mouse models with the goal of establishing the rationale for translation of preclinical findings to pre-Phase I or Phase I clinical studies. Currently CAPR supports intramural research conducted at CCR's laboratories led by Dr. Glenn Merlino, Dr. Udo Rudloff, Dr. Ira Pastan, Dr. Christine Alewine, Dr. Brad St. Croix, and Dr. James Doroshow. CAPR also contributes to extramural collaborations with the Division of Cancer Prevention (NCI) and NCATS (Dr. Craig Thomas) to evaluate novel therapeutics in a mouse model of ovarian cancer.

CAPR has continued the extension of its external partnership portfolio by initiating new projects with nonprofit and industry organizations, while at the same time broadening the scope of its previously established collaborative activities with the existing partners. Several CRADA projects are currently underway. A new CRADA was signed between CAPR and SolaranRx to evaluate SolaranRx's proprietary radiolabeled peptide (SRX-1177) in preclinical mouse models of metastatic melanoma. The first part of the study, evaluation of SRX-1177 uptake and biodistribution in normal and melanoma tissue, has been completed and the first milestone payment has been received. The second part, evaluation of SRX-1177 efficacy in a melanoma tumor model, will be completed this year. CAPR also entered into a collaborative agreement with the University of California at San Francisco (Dr. Frank McCormick's lab) with the goal of examining the clinically approved antiretroviral therapeutic compound prostratin in cell culture models, with the aim of repurposing this drug for treatment of pancreatic cancer. As a continuation of the collaborative agreement work scope, CAPR intends to pursue a CRADA-supported partnership to also assess prostratin in vivo efficacy in a panel of murine pancreatic cancer models. Additionally, CAPR is working in partnership with MedImmune (under CCR's umbrella CRADA) to evaluate MedI's immune checkpoint inhibitors in metastatic melanoma. CAPR has already completed several immune checkpoint inhibitor efficacy and biomarker studies in a melanoma mouse model, and presented the results at the joint NCI-MedI steering committee meetings. Finally, CAPR has signed a CRADA to partner with AstraZeneca to evaluate therapeutic combinations of targeted therapeutics with immune checkpoint inhibitors in a mouse model of serous epithelial ovarian cancer.

CAPR has expanded existing cCRADA projects, with the addition of three new TSA modules to the current roster of preclinical projects with AbbVie, Inc., that pursue in-depth evaluation of novel drug candidates. Further outreach efforts have also yielded a novel TSA with Inception 2, Inc., to conduct a therapeutic assessment of the company's proprietary compound NXT1120 as a single agent, or in a combinatorial intervention regimen against pancreatic cancer.

Support Provided by the Data Science and Information Technology Program

Genetics Branch

The mission of the Genetics Branch (GB) is to discover, diagnose, investigate, and treat genetic aberrations that form the bases for oncogenesis. To this end, the GB performs basic research on genetic instabilities that drive cancer and provides clinical services, including risk screening, education, counseling, genetic testing, and supports translational research, on molecular diagnostics, genotype/phenotype correlations, biomarker discovery for risk, prognosis and therapeutic response, and drug discovery.

ABCC/DSITP personnel are embedded in the GB laboratories to provide dedicated bioinformatics, wet-lab, and managerial support. In the past year, ABCC personnel have supported three main efforts within the GB: infrastructure development, genomic data sharing, and the ClinOmics precision medicine initiative.

In the past year, ABCC analysts have completely revamped and modernized the GB's flagship application, the Oncogenomics web portal. Oncogenomics allows users to query, analyze, and visualize sequence data generated in the Khan lab. The underlying database can store diverse types of sequence data generated from both microarrays and next-generation sequencers. The database currently stores, and allows access to, eight whole genome, 1,732 exome, 658 Panel, and 1,225 RNA-seq samples. The application has diverse ways of querying, filtering, and visualizing both sample-level and gene-level data. Several data analysis methods have been included: principal components analysis, correlation, gene-set enrichment analysis, and Kaplan-Meier curves.

A second major infrastructure development initiative in the past year has been the ChIP-seq data analysis pipeline, which has been developed to facilitate the analysis and interpretation of ChIP-Seq experiments conducted within the GB. The pipeline has several useful features: batch peak calling; peak annotation, summary, and comparison; nucleosome-free regions calling; motif finding; and super enhancer finding. The pipeline can also integrate ChIP-seq data with related RNA-seq data. The ChIP-seq pipeline has been used to produce data for several publications [1,2,3].

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Genomic Data Sharing

The NIH released its Genomic Data Sharing (GDS) policy in August 2014 to promote data sharing as a way to facilitate the translation of data into knowledge, products and practices that improve health while protecting the privacy of research participants. The GDS policy applies to all NIH-funded research that generates large-scale human and non-human data, irrespective of funding level or funding mechanism. Large-scale data include data from genome-wide association studies (GWAS) and single nucleotide polymorphism (SNP) arrays, as well as genome sequence, transcriptomic, metagenomic, epigenomic, and gene expression data. In addition, any study metadata that are necessary to reproduce any published table or analyses, or that are pertinent to the interpretation of the genomic data, are expected to be shared. ABCC personnel worked collaboratively with senior CCR leadership to implement the GDS policy within CCR's clinical program. This included: the development of processes, standard operating procedures, informational websites; outreach sessions to educate CCR staff on the policy and its expectations; compliance audits of studies that fall within purview of GDS; and the development of infrastructure that facilitates the data submission and registration process and the process of compliance tracking. GDS efforts, in this time period, were broadly focused on policy education, audit, and infrastructure development. Branch-level meetings were held at Dermatology, Vaccine, Neuro-Oncology and Urologic Oncology to educate PIs on the expectations of GDS policy and the process of genomic data sharing. In addition, an introduction to GDS and the current level of compliance to GDS, within CCR, was presented to the recently appointed CCR director, Dr. Tom Misteli.

We performed a quarterly audit of all open clinical studies in CCR. Manual and automated audits were performed to identify GDS applicable studies and assess their level of compliance. Branch-wide compliance reports were compiled from our audit findings to be shared with the respective Branch Chiefs. A quarterly report was also sent to the Director of the CCR.

To facilitate use and ease the burden of policy compliance, we are developing a web-based portal that enables the submission of key GDS documents and tracking of GDS applicable projects. A web-based Genomic Data Sharing Plan (GDSP) submission portal (https://service.cancer.gov/ccrgdsp/) has been developed that reduces the burden of submitting, reviewing, and storing these documents. PIs can fill in and submit their GDSPs online. Reviewers, the Genomic Program Administrator (GPA), and the Scientific Director can review and provide feedback through the online portal. The portal also allows PIs to resubmit, in case reviewers need modifications or if plans change over time, and to store plans long-term. The web-based portal was developed in collaboration with CBIIT. In addition, we have developed a tracking system that allows the GPA to track compliance of genomic data generating projects across the approximately 200 labs within CCR. The long-term plan is to merge the submission portal with the tracking system to further streamline the genomic data sharing workflow.

ClinOmics

The ClinOmics Program was established by CCR leadership to harness modern tools and technologies to develop a state-of-the-art comprehensive translational genomics platform to enable precision therapy trials for all patients (both pediatric and adults) at the NIH Clinical Center for NCI. The overall goal of the program is to introduce an individualized, molecular dimension to the interpretation of clinical trial results. ABCC/DSITP supports the objectives of the ClinOmics program by providing both wet-bench and bioinformatics support. The ClinOmics data analysis pipeline has been stabilized and is currently undergoing FDA review. The server environment has been generating diagnosis quality reports since early 2016. The sequencing data processing (retrieving/de-mux/distribution/notification/backup) pipeline has been automated and is in production mode. ABCC analysts have installed and configured a LIMS system for ClinOmics; it is currently in user training/beta testing phase. Finally, ABCC analysts have developed an online tool, NGBarcode, that assures sufficient sequence barcoding diversity in the preparation of multiplexed NGS libraries. In addition to IT support, ABCC/DSITP has currently dedicated one bench scientist to completing the pilot phase of the ClinOmics program. In the long term, ABCC/DSITP plans to increase its footprint within ClinOmics to meet the evolving and increasingly diverse needs of the program.

Radiation Oncology Branch

The mission of the Radiation Oncology Branch (ROB) is to conduct preclinical and clinical research on the biological and therapeutic effects of radiation administered to cancer cells, either alone or in combination with other therapies. ROB also provides radiation therapy and medical consultation to patients admitted to the Clinical Research Center clinical services.

ABCC/DSITP personnel are embedded in ROB laboratories to provide dedicated wet-lab and bioinformatics support. In the last year, ABCC personnel have taken part in two main projects: (a) studying the interaction of novel drugs and radiotherapy in the treatment of glioblastoma multiforme (GBM) using preclinical model systems and (b) developing a web resource for discovering and understanding synthetically lethal interactions specific to human cancer.

The GBM studies are presently aimed at understanding the mechanisms mediating radiation-induced translational control of gene expression and whether radiation therapy provides a source of targets for tumor-specific radiosensitization. Building on earlier work [1] that identified MPS1 as a radio-sensitizing agent in GBM, the ROB team demonstrated that the inhibition of MPS1 leads to the altered expression of genes

associated with neurological disease, nervous system development, DNA replication, recombination, and repair pathway, thus providing a molecular basis for the enhanced radiosensitivity exhibited by GBM cells after MPS1 inhibition [2]. Additional studies on the radiobiology of GBM specifically address the influence of the brain microenvironment, using GBM stem-like cells isolated from human surgical specimens grown in vitro and as intracranial xenografts.

The "synthetic lethality" web resource is being built as a repository for diverse types of biological data. It contains over 150 million records consisting of various data from gene, protein, and drug annotations; gene expression data; mutation data; gene–gene/protein–protein interactions; drug activity data; and drug–target and gene–disease associations. The application is being developed to provide scientists and clinicians access to diverse biological data sets through an integrated and easily navigable interface. The application is projected to be ready for public release by early next year.

References

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2. Shankavaram, U., et al., Molecular profiling of MPS1 gene silencing in U251 glioma cell line. *Genom Data*, 2015. 6: p. 36-39.

HIV Dynamics and Replication Program

Support of the HIV Research Program

While significant progress has been made in reducing the HIV viral load in affected individuals using anti-retroviral therapy (ART), drug resistance has been identified, and these infected cells are able to replicate. They are referred to as expanded clones. The SAMM group provides support for the NCI HIV Dynamics and Replication Program, particularly the Translational Research Unit (TRU) and Clinical Retrovirology Section (CRS) and their collaborators around the world. We recently collaborated in a study which found that certain expanded clones became infectious if ART was stopped (Simonetti et al., *Proc Natl Acad Sci USA*. 2016 Feb 16;113(7):1883-8).

To identify and characterize expanded clones, the TRU and CRS have developed procedures for single-genome sequencing (SGS). The SAMM group developed computational workflows to assemble SGS results and to align different assemblies to determine if one or more expanded clones are present. The Miseq sequencing technology is also a very powerful tool used in HIV studies. TRU frequently uses Miseq to study HIV drug resistance, evolution, and persistence. The SAMM group developed computer programs to analyze the data and provided data analysis for TRU and collaborators at the University of Pittsburgh.

Numerous studies on retrovirus integration into the host genome have been published. However, there is no public database to store the published data. We constructed the NCI Retrovirus Integration Database to store published and submitted integration information so that the data can be made publically available (Shao et al., *Retrovirology* 2016 Jul 4;13(1):47).

CCR Flow Cytometry Core

The ABCC/SWPG supports the CCR-Frederick Flow Cytometry Core by providing a scheduling tool system that allows users to reserve analyzer instruments to design experiments that will yield reliable data. The advanced scheduler allows multiple schedules to be created and managed across numerous instruments, users, and directorates, and it allows directorates to share these resources. Robust notifications and search features provide up-to-date information on instrument availability. The schedule also updates in realtime so that, as a user creates a reservation, other users can see the new events as they happen. Released in early 2016, the initial schedule supports 50 users and 8 instruments for the CCR-Frederick Flow Cytometry Core as their single scheduling resource. Plans are in place to add additional directorates during FY2016.

CCR Sequencing Facility

The Center for Cancer Research Sequencing Facility Bioinformatics Group (CCR-SF IFX) provides next-generation sequencing (NGS) and bioinformatics analysis support to CCR investigators across NCI. Specialized in NGS data analysis and quality control, CCR-SF IFX provides several services including sequencing technology consultation, experiment design, data analysis and management, and results interpretation. The group has established standard workflows and pipelines to process the rapidly growing volume of sequencing data and has worked closely with investigators alongside their projects to deliver high-quality data and reproducible analysis results in a timely manner.

Sequencing requests have increased at a growing rate each year since the inception of CCR-SF in 2009. The CCR-SF bioinformatics team has provided services to 87 laboratories and branches across CCR as well as from collaborator laboratories within NIH. This year, CCR-SF has received 295 sequencing requests and processed over 4,700 samples from various sequencing applications—a greater than 20% increase from the previous year. CCR-SF IFX has successfully processed all project requests with a fast turn-around time while maintaining top industry-quality control standards and has delivered more than 40 trillion base pairs of pass-quality-control data to CCR investigators.

In addition to production support, CCR-SF IFX has also actively engaged in technical research and development. In order to provide investigators access to the latest NGS technologies, a continuous effort has been made at both the technical and informatics level towards

developing and adopting additional NGS pipelines. Several new and exciting emerging NGS technologies have been tested and made available to the CCR research community in 2016. The following are the highlights of some of the key accomplishments.

Phased Sequencing and Large Structural Variation Detection Using 10x Genomics Technology

Structural genomic changes (and particularly gene fusion events) are known to be the driving mutations in many forms of cancer. With the current short-read sequencing technologies, it is challenging to detect complex structural rearrangement and suffer limitations to detect low frequency events. 10x Genomics combines microfluidics technology and Illumina sequencing to individually barcode high-weight DNA molecules, generating linked reads that preserve haplotype information and allow the detection of complex structural variations.

In order to bring this technology from the development stage into a full production environment, CCR-SF IFX has worked closely with lab scientists to validate several 10x Genomics protocols, including whole-genome and exome pipelines for phasing genetic variation (SNVs, indels), resolving large structural variation, and detecting other valuable long-range information. CCR-SF IFX has extended the existing 10x Genomics analysis pipeline to perform additional functions, such as sequencing read barcode tagging and counting, de novo assembly of the linked reads, and copying number variation detection. The group has completed several pilot projects for using 10x Genomics pipelines. As an example, the group successfully applied this technology to detect and deconvolute the structure of Merkel cell polyomavirus integration sites in human samples, an important goal in understanding the origin of cancer within these infected tissues. With the forthcoming upgrades to 10x Genomics technology in the form of the Chromium platform (e.g., increased detection sensitivity, reduced sequencing coverage cost, and the ability to sequence single-cell RNA samples), this platform will provide an even wider range of novel applications.

DNA Methylation Protocols Validation and Analysis

DNA methylation has an important impact on normal cell physiology and serves as a critical player in transcriptional regulation, chromatin remodeling, embryonic development, and the cellular differentiation process. Currently, there are several NGS technologies used for detecting cytosine methylation, including whole-genome bisulfite sequencing (WGBS), target Methyl-seq, Reduced Representation Bisulfite Sequencing, and Oxidative Bisulfite Sequencing. In addition, Single Molecule Real Time Sequencing directly detects epigenetic modifications by measuring kinetic variation during base incorporation. In order to provide cost-effective protocols with improved detection sensitivity and accuracy to the CCR research community, the CCR-SF team has tested and compared four different methylation detection kits this year. CCR-SF IFX has worked closely with CCR-SF scientists and vendors to develop and integrate software pipelines that support methylation analyses. Based on comparison analysis results, the group has established standard QC metrics and placed the high-performing kits into production.

Iso-Seq Whole Transcriptome and Targeted Full-Length Transcript Analysis

The PacBio Isoform Sequencing (Iso-Seq) generates full-length cDNA sequencing without the need for assembly, isoform inference, or other reconstruction algorithms typically required by short-read sequencing technologies. In the human genome, the large majority of genes are alternatively spliced, and there are many isoforms specifically associated with cancer progression and metastasis. Iso-seq technology enables the direct sequencing of full-length transcripts, allowing structural transcript variations to be directly observed and studied. CCR-SF IFX has worked collaboratively with both the CCR-SF laboratory and Genomic Laboratory scientists, identified several limitations of the standard protocol, optimized the protocol to address issues such as reverse transcriptase (RT) skipping due to secondary structures, and developed a method to normalize and reduce the prevalence of highly expressed transcripts in order to obtain a higher resolution of rare transcripts. A new pipeline for targeted Iso-seq isoform structure clustering and transcript counting has been developed by the CCR-SF IFX team. The targeted Iso-seq method has proved to be a cost-effective way to study alternative splicing and identify novel transcripts for genes of interest. CCR-SF IFX has provided data analysis services to over a dozen laboratories utilizing Iso-seq this year. As an example, the team provided data analysis support for a study from the Pediatric Oncology Branch using a combination of Iso-seq, RNA-seq, and exome-seq to survey the genomic landscape of leukemia under the selection pressure of CAR T-cell Immunotherapy. Using the Iso-seq method, the group has found a higher-than-expected degree of heterogeneity in the target gene isoforms, including a novel isoform that may have implications for resistance to relapse after the targeted gene immunotherapy.

Single-Cell Whole Transcriptome Analysis

Single-cell RNA-seq has gained great popularity in recent years, enabling high-throughput and high-resolution transcriptomic analysis of individual cells. More recently, the focus has shifted toward innovative techniques for efficiently handling large numbers of cells. There are several technologies available, including droplet microfluidics-based systems, that enable the comprehensive study of subpopulations of cells with heterogeneous populations. CCR-SF, in collaboration with the Genomics Lab and several laboratories within NCI, is actively testing several single-cell applications/protocols, such as Drop-Seq whole transcriptome 3’mRNA counting, Pacbio sequencing of

full-length transcript from modified Drop-seq protocol, and 10x Genomics Single-cell 3' mRNA counting method. The CCR-SF IFX has performed the analyses for the pilot studies. In addition, the group has compared different single-cell RNA-seq analysis pipelines and currently is developing an in-house pipeline that integrates the different methods, streamlines the analysis process, and provides standard reports.

Other Accomplishments and Ongoing Efforts

- Expanded informatics workflows and data analysis pipelines to support new NGS technologies. Several new data analysis pipelines were developed by the CCR-SF IFX team, including structural variation detection, de novo assembly (both long reads and short reads), methyl-seq subtraction analysis for detecting a specific type of modified bases, RNA-seq analysis for low-input and low-quality FFPE samples.
- Ongoing LIMS development allowing for improved query and reports functionality and for providing information-sharing capabilities between systems.
- The CCR-SF IFX has worked closely with the CCBR bioinformatics group for the development of best practices in NGS data analysis. The two groups have formed joint working groups for the software pipeline development for exome-seq, RNA-seq, Chip-seq and miRNA-seq data analysis. The software pipelines developed will be shared with the CCR community as well as the large open-source community.
- Ongoing collaboration with the HPC group at DSITP to develop a centralized data repository for genomic data and metadata storage and distribution.

CCR Collaborative Bioinformatics Resource (formerly CCRIFX Bioinformatics Core)

The CCBR (https://bioinformatics.cancer.gov/) was established in 2014 as an umbrella organization to provide collaborative bioinformatics support to investigators across CCR. The CCBR provides single-point access to a broad range of bioinformatics expertise that exists across several bioinformatics groups within NCI. The CCBR group includes members of Leidos Biomed (LBR-CCBR team), the CCR Office of Science and Technology Resources (OSTR), FNLCR, and the CBIIT. The CCBR also serves as a central hub to direct projects to other available CCR bioinformaticians as needs demand.

The LBR-CCBR team comprises of two key ABCC groups that have been subsumed within the CCBR: the CCR Informatics Core (CCRIFX) and the Basic Science Program (BSP)-CCR Genetics Core (BCGC). The CCRIFX was set up in 2011 as a shared bioinformatics support group for the CCR investigators. The BCGC originated as an embedded core of the Laboratory of Genomic Diversity, a leading laboratory in research on population genetics and evolution.

In the last year, the LBR-CCBR team has worked on approximately 134 analysis requests (complete and ongoing) that were submitted from across 57 programs, branches, and labs. The analysis requests addressed a wide spectrum of questions in cancer research, ranging from basic biology to clinical applications. The requests typically involved the processing, analysis, and interpretation of high-dimensional data sets generated by microarray, Exome-seq, RNA-Seq, ChIP-seq, metagenomics, and mass spectrometry platforms, as well as publicly available data.

In addition to providing bioinformatics support, the LBR-CCBR has actively engaged in technical development (Tech-Dev) projects to keep abreast in the evolving field of biological data analysis. The Tech-Dev projects are aimed at the development of best practices in NGS data analysis. Best practices have been developed for analysis of exome-seq, RNA-seq, ChIP-Seq, miR-Seq, and microarray datasets. As part of our Tech-Dev efforts in developing tools for genomes-scale data generation and analysis, we also participated in the PrecisionFDA Truth challenge, which allowed for direct benchmarking and competitive comparison of genome assembly pipelines from groups throughout the United States. The overarching goal of these community challenges is to "assess and improve the techniques used in DNA testing," and the Truth challenge sought specifically to optimize exome-seq pipeline performance. To achieve this, the FDA provided one extremely high-quality genome with a known and verified truth set of variants, and participants assembled the exome and compared their results to this truth set. In addition, a second genome ethnically diverged from the first test genome and, with its truth set not publicly available, was provided for comparison. Performance was assessed by comparing recall, precision, and reproducibility. In this challenge, we performed exceptionally well, with our precision ranking in the top five of approximately 40 submissions on the first test genome, and in the top 10 of all submissions on the second. For all comparisons, we achieved 99.9 percent variant recall and 99.5 percent precision, suggesting our pipeline is exceeding the performance of most other benchmarked pipelines. In addition, we have been utilizing the FDA truth sets and benchmarking tools to attempt to further improve our pipelines and understand the genomic context within which genotyping errors occur, with the ultimate goal of developing new variant detection approaches that minimize errors and maximize sensitivity.

One significant initiative undertaken by the team this year is the implementation of robust, scalable, and flexible scientific analysis pipelines for analysis of exome-seq, RNA-seq, ChIP-Seq, miR-Seq, and microarray datasets. The software pipelines have been designed to perform the semi-automated processing of large data sets and generate shareable result files. They reduce total data processing time, ensure uniform data processing standards across the team, reduce manual hands-on time, and reduce the probability of manual

errors. Documented and version-controlled pipelines are being shared with the CCR community via the CCBR-GitHub site.

The CCBR team collaborates with the CCR Sequencing Facility to keep abreast of new sequencing technologies. A team of CCBR analysts are involved with the implementation and testing of analysis workflows for PacBio long-read sequencing, single-cell RNA sequencing, and whole-genome sequencing technologies.

The CCBR holds an annual workshop series, the Bioinformatics Training and Education Program (BTEP), which is designed to educate and empower researchers who lack the necessary computational skills in processing, analyzing, and interpreting their data according to state-of-the-art analysis protocols. In the past year, LBR-CCBR members participated in seven hands-on monthly workshops to educate the CCR research community on a diverse range of topics that included programming in R, TCGA data integration, concepts and analysis of microarray data, as well as the three most commonly utilized Next-Generation Sequencing techniques (exome-seq, ChIP-Seq, and RNA-Seq).

The CCBR's activities impact both the CCR research community and the state of science. At the community level, the CCBR has been instrumental, through its Tech-Dev and BTEP sessions, in developing and transmitting to the CCR researchers sound and rational data-analysis practices that represent the latest advances in bioinformatics and are accepted by the wider research community. The CCBR's impact on the state of science is best illustrated by the following selected accomplishments by the LBR-CCBR analysts in the past year:

- Triple negative breast cancers (TNBCs) account for 10–15 percent of all breast cancer cases, are characterized by inferior survival outcomes, and remain difficult to treat using standard treatment. This cohort may benefit from developing novel patient stratification strategies. The CCBR team collaborated with the Women's Malignancies Branch (WMB), which conducts research in cancers occurring in women, and links experimental cancer biology to clinical investigation. The bioinformatics analysis support provided clinical context to in-vitro data generated by the lab that showed tumor necrosis factor (TNF)¬–related apoptosis-inducing ligand (TRAIL) preferentially killing the mesenchymal subtype of TNBC through activating death receptor 5 (DR5). The purpose of this study was to identify potential biomarkers of drozitumab-sensitive breast cancer cells and determine if those biomarkers were present in tumors from patients with TNBC. CCBR assisted with the data mining of publicly available gene-expression profiles of relevant breast cancer datasets. The analysis focused on the transcriptomic profiles of two genes, vimentin and Axl, and their correlation to overall survival, with a particular emphasis on mining a subset of African-American women with TNBC. The inference from this analysis by CCBR, along with other results, supported that vimentin and Axl may be useful to identify TNBC patients who would be most likely to benefit from a DR5 agonist (PMID: 26759246).
- RNA polymerase II (PolII) regulation has been a central mechanism of cellular responses to environmental changes, and misregulation of RNA expression is fundamentally linked to the formation of cancers. One of the recent major breakthroughs in the RNA PolII regulation is the discovery of paused polymerases and their releases at the promoter sites of genes. The CCBR team collaborated with Dr. Brian Lewis to understand the role of O-GlcNAc in RNA polymerase pause and release mechanisms by analyzing ChIP-seq and microarray data in various cell lines. Our analyses showed that O-GlcNAc is part of RNA PolII pause-release regulation and possibly a nutrient-sensing mechanism of PolII. (Melissa Resto, et al. O-GlcNAc Aminidase is an RNA Pol II Elongation Factor Coupled to SPT5 and TRIM28/KAP1/TIF1b, manuscript in preparation)
- Treatment of refractory Pre-B Acute Lymphoblastic Leukemia with CD19 chimeric antigen receptor (CAR) has demonstrated impressive results in multiple clinical trials. However, PD1+ T cells from leukemic mice fail to protect against E2a-PBX1.3 following adoptive transfer. Furthermore, expression of a CAR-targeting CD19 in T cells from leukemic mice fails to restore function. The CCBR team collaborated with Dr. Terry Fry's lab to analyze the gene expression signatures in CAR T cells derived from naïve and tumor-bearing mice and showed that genes down-regulated in tumors are involved in pathways such as T-cell suppression, energy depletion, as well as hypoxia. (Haiying et al. manuscript is prepared for submission to J Clin Oncol)
- HPV is known as a causative agent of cervical cancers. However, molecular studies of HPV have been rather difficult and time-consuming, mostly due to the lack of a popular murine model system like mouse or rat. In collaboration with Dr. Zhi-Ming Zheng, CCBR analysts analyzed RNA-seq samples of mice infected with recently characterized murine papillomavirus to understand its effect on host gene expression and its integration of host genome. (Construction of a full transcription map of mouse papillomavirus type 1 from wart tissues. Xiangyang Xu et al. manuscript in preparation)
- Familial non-medullary thyroid cancer (FNMTC) accounts for 3–9 percent of all thyroid cancer cases and exhibits an autosomal-dominant pattern of inheritance. CCBR, in collaboration with the Endocrine Oncology Branch (EOB), previously identified the gene HABP2 as a susceptibility locus for a single family, and subsequent analyses of additional families indicated distinct loci for each family and therefore very little genetic overlap. To pursue this hypothesis and identify additional FNMTC susceptibility loci, the team is currently

analyzing 143 exomes from 31 additional families. In addition, we have initiated a multicenter international collaboration analyzing many more FNMTC families in addition to the 31 already underway.

- In the pursuit of therapeutic targets for sporadic-origin cancers, a critical component is understanding the mutational processes underlying the biological progression of the tumor. As part of this effort, CCBR and the EOB are working to analyze large tumor/germline datasets for several cancer types. Specifically, analyses of a large set of adrenocortical carcinoma (ACC) metastases have identified sites of mutations common to both metastases and primary tumors, as well as mutations unique to metastases and therefore representing potential metastasis-specific therapeutic targets. In parallel, we are analyzing a large tumor/germline insulinoma cohort to identify the major genetic events that drive primary tumorigenesis; functional confirmation of somatic variants is currently under way. As an additional component of our FNMTC analyses, we are also analyzing a large set of tumor/germline samples to understand the relationship between germline risk factors and the mutational landscape of the resulting tumor.
- To understand how mutation drives cancer progression, it is necessary to characterize the mutational landscape through both space and time (from early transformation through metastasis). In a collaboration with Dr. Robert VanderWeele's lab, we are analyzing mutations in the primary tumor, metastases, and circulating tumor cells (CTCs) for two prostate cancer patients, which involves the challenging analysis of single-cell CTC exomes. Thus far, we have mutationally characterized each of these samples for both patients, and mutations are currently being analyzed to identify events important for each of the following stages: primary tumorigenesis, initial metastasis, and subsequent metastasis via CTCs. The outcome of this analysis will provide a basic understanding of the mutations and genes crucial to the progression of prostate cancer from initial tumorigenesis to metastatic disease.
- An invaluable tool in the study of cancer is the development and characterization of disease models. In this study, we attempted to characterize the mutational landscape of the mouse lung adeno-carcinoma models KRAS+/- and KRAS+/-/chuk+/- by calling single nucleotide and insertion/deletion variants, in addition to calling large-scale structural variants. We called somatic variants for three replicates of each model (KRAS+/-, KRAS+/-/chuk+/-), attempting to correct for the challenges of non-paired tumor/germline samples by subtracting all known strain-specific mutations. Both somatic mutation and structural variants suggest mutation rates are significantly higher for the KRAS+/-/chuk+/- model, and the KRAS+/- model mutational landscape is largely dominated by structural variation (amplification and deletion at the chromosome level). Future data generation is underway to confirm these patterns and to illustrate the mutational consequences of KRAS mutation in lung adenocarcinoma progression.
- Splicing factor mutations (mutation in U2AF35, SF3b1, SRSF2, etc.) have been recently identified as a novel class of gene alterations in cancers, but their contribution to the pathogenesis of hematopoietic malignancies is not known. CCBR, together with the Laboratory of Receptor Biology and Gene Expression, performed Nascent-seq analysis, which unveiled increased density of reads mapped to the intergenic regions and upregulated intergenic transcription in the cells with the U2AF35 S34F mutation. Also, it was demonstrated that aberrant intergenic transcription is rescued in the cells with frameshift alleles. This analysis provides insights into how mutations affect the splicing process, transcription, chromatin and nuclear organization, and how cancer cells benefit from the mutations in the splicing machinery.
- Lens epithelium-derived growth factor (LEDGF) fusion proteins can direct HIV-1 DNA integration to novel sites in the host genome. LEDGF normally directs integrations to the bodies of expressed genes. Replacing the N-terminus of LEDGF with chromatin binding domains (CBDs) from other proteins changes the specificity of HIV-1 DNA integration. Currently, CCBR is performing analysis of available datasets with 10,000–20,000 insertions to uncover the distribution of hits with respect to different genomic regions and relative insertion location, as well as insert density across the genome. The ability to redirect HIV-1 DNA integration may help solve the problems associated with the activation of oncogenes when retroviruses are used in gene therapy.
- Many anti-cancer drugs currently used to target DNA, such as camptothecin and doxorubicin, are considered to be DNA-breaking agents. However, more and more data indicate that these drugs are in fact chromosomal structure modifiers, with the extreme results of DNA breaks. In collaboration with the Developmental Therapeutics Branch (DTP), CCBR implemented Assay for Transposase-Accessible Chromatin next-generation sequencing (ATAC-seq) technology to monitor the DNA structural changes upon drug treatments, with the focus on SLFN11-related chromosome structural changes upon camptothecin treatment.
- Together with the Laboratory of Cellular Oncology (LCO), CCBR is aiming to develop and perfect a single-cell ChIP-seq technology. Existing methods do not allow detection of rare cell populations (less than 5 percent), and are unable to detect peaks in individual cells without data aggregation because of sparse coverage (around 2,400 reads per single cell). Efforts are underway by the CCBR team to fine-tune the procedure and provide reliable estimates for noise and background.

- Effective treatment of prostate cancer depends on controlling the progression of the disease from the prostate to other parts of the body. However, the mechanisms of transformation from benign to metastatic prostate tumors are not well understood. In an attempt to understand the evolution of tumor cells taken from multiple regions of the prostate, tumor biopsies from up to 10 regions of the prostate were taken from 10 subjects (90 samples in total), genotyped for prostate cancer–related SNPs, and subjected to low-pass whole-genome sequencing (WGS). The CCBR team analyzed WGS sequence data to identify regions of copy number variation. The resulting CNV data was combined with the SNP data (generating a bit array) and processed using a program called Network to produce, for the samples arising from each of the 10 subjects, a map relating each sample's bit array to the others via a route requiring a minimal number of bit changes per transition. The resulting maps were diverse in topography across the 10 subjects, suggesting no common pattern of SNP and CNV accumulation. However, some correlation between the proximity of the samples taken from a single tumor to one another and the similarities of their genotypes was observed.
- Pancreatic neuroendocrine tumors (pNETs) are a type of neuroendocrine tumor that accounts for 1–2% of pancreatic cancers. PNETs comprise several subtypes, and optimal treatment depends upon subtype recognition. In a collaboration with Dr. Xavier Keutgen's lab, miRSeq count data from 33 pancreatic cancers was analyzed with the goal of discovering assayable markers for three major pancreatic cancer subtypes: VHL (characterized by mutations in the von Hippel-Lindau tumor suppressor gene), multiple endocrine neoplasia type 1 (MEN1), and Sporadic. The analysis generated sets of miRs that were differentially regulated between each of the tumor subtypes and the normal. The majority of the VHL-specific miRs were subsequently validated as overexpressed in VHL and are potential candidates for diagnostic markers. These results served as preliminary data supporting hypotheses for two recent grant proposals submitted by the PI.
- The integrated stress response (ISR) controls cellular adaptations to nutrient deprivation, redox imbalances, and endoplasmic reticulum (ER) stress. ISR genes are upregulated in stressed cells, primarily by the bZIP ATF4 through its recruitment to cis-regulatory C/EBP:ATF response elements (CAREs) together with a dimeric partner of uncertain identity. CCBR collaborated with Dr. Peter Johnson's lab in the Mouse Cancer Genetics Program (MCGP) to show, using integrative analyses of ChIP-Seq and RNA-Seq data, that C/EBPγ:ATF4 heterodimers, but not C/EBPβ:ATF4 dimers, are the predominant CARE-binding species in stressed cells. C/EBPγ and ATF4 associate with genomic CAREs in a mutually dependent manner and coregulate many ISR genes. In contrast, the C/EBP family members C/EBPβ and C/EBP homologous protein (CHOP) were largely dispensable for induction of stress genes. These and other related findings identify C/EBPγ as a novel antioxidant regulator and an obligatory ATF4 partner that controls redox homeostasis in normal and cancerous cells (PMID: 26667036).
- The ancient, highly conserved, Wnt signaling pathway regulates cell fate in all metazoans. Dr. Terry Yamaguchi's lab, Cancer and Developmental Biology Laboratory, has previously shown that combined null mutations of the specificity protein (Sp) 1/Klf-like zinc-finger transcription factors Sp5 and Sp8 (i. e., Sp5/8) result in an embryonic phenotype identical to that observed when core components of the Wnt/β-catenin pathway are mutated. However, their role in Wnt signal transduction is unknown. Through an integrative analysis of RNA-Seq and ChIP-Seq data, we showed in mouse embryos and differentiating embryonic stem cells that Sp5/8 are gene-specific transcriptional coactivators in the Wnt/β-catenin pathway. Sp5/8 bind directly to GC boxes in Wnt target gene enhancers and to adjacent, or distally positioned, chromatin-bound T-cell factor (Tcf) 1/lymphoid enhancer factor (Lef) 1 to facilitate recruitment of β-catenin to target gene enhancers. Because Sp5 is itself directly activated by Wnt signals, we propose that Sp5 is a Wnt/β-catenin pathway-specific transcription factor that functions in a feed-forward loop to robustly activate select Wnt target genes (PMID: 26969725).
- Discovery of novel prognostic biomarkers is very important to identify high-risk cancer patients. Glioblastoma multiforme (GBM) is an aggressive brain tumor with a five-year survival rate of less than 10 percent. It is more common in males, but the molecular basis of this gender bias is not understood. To this end, and with collaboration with Dr. Karlyne Reilly, CCBR analysts looked for genes that have a gender-specific effect on GBM survival using two microarray expression datasets from TCGA. Based on pathway enrichment analysis, the study suggested that females with high gene expression for the proneural signature have better prognosis compared to females with lower expression, while males with high expression for the mesenchymal signature have better survival. Focusing further on a subset of the dataset processed at University of North Carolina, a gender-specific prognostic signature was discovered that is independent of subtype, which may lead to a better understanding of disease progression in GBM. This study was presented in a poster at the ISMB 2016 conference and will lead to a future publication.
- Identifying kidney germline variants and analyzing their effects on somatic mutations is very important for kidney cancer disease development. To achieve

this end, we are analyzing TCGA kidney exome-seq samples in collaboration with Dr. Marston Linehan's team. The analysis carried out so far has helped identify new high-impact variants that may affect individual sensitivity to tumor development. The outcome of this study is very relevant to understanding kidney cancer development.

- HIV-associated nephropathy (HIVAN) is common among African Americans in the absence of effective therapy, but rarely, if ever, seen in European Americans, pointing to an African inheritance. A mapping by admixture linkage disequilibrium (MALD) analysis by BCGC analysts identified an extremely strong association with a region of chromosome 22. Further research focused this association on two protein-altering variants in the APOL1 gene. This is the most significant genetic association with kidney disease, applying to many conditions beyond HIVAN, and one of the strongest genetic associations with any common disease.
- The Noble rat is a model for several important human cancers and, in particular, is subject to a nephroblastoma analogous to Wilms' tumor. The standard lab Fischer rat is not susceptible. The Cancer and Developmental Biology Laboratory (CDBL) has an ongoing effort to map susceptibility loci for this tumor using backcrosses between Noble and Fischer. BCGC analysts provided the experimental design for the current mapping effort, specifying a backcross of hybrid rats to Fischer. (The alternative backcross provides three times as many tumor-bearing rats, but each provides less than one-third the information.) For this project, they devised and are testing a novel method of mapping by very-low-coverage sequencing. Previous experiments used an Affymetrix rat genotyping chip, which is no longer manufactured. The analysts considered that, starting from high-coverage sequencing of the parental inbred Noble and Fischer strains, very-low-coverage sequencing of the backcross rats would identify enough variant SNPs distinguishing the strain to provide a high-coverage map; the standard approach of a custom genotyping chip would be more expensive and provide a less dense map.
- Variation in the human leukocyte antigen (HLA) region is the strongest host genetic factor affecting AIDS. The laboratory of Dr. Mary Carrington has played a key role in characterizing this influence, starting with a seminal 1999 paper describing the effect of HLA class I homozygosity and the B*35 allele on AIDS progression. A current focus is the effect of allele differences in expression for HLA Class I alleles on AIDS and autoimmune disease. We have previously shown that HLA C expression is associated with resistance to HIV disease progression, while on the other hand associated with susceptibility to autoimmune disease. We are looking for further associations with Class I expression levels, by themselves and in association with different alleles of the killer immunoglobulin-like receptors (KIRs) of natural killer cells. A new result is that the resistance to AIDS progression conferred by HLA C expression is primarily carried by one subset of C alleles, the C1 alleles; for the C2 alleles the effect is weaker, probably because it is offset by an interaction with KIRs specific to this set of alleles. These analyses require a sensitive regression to predict expression levels of different alleles; the experimental data is for overall expression of locus for an individual, in general carrying two alleles, and the power of the association analysis requires an optimum estimate of the expression of each allele, averaged over the individuals tested. We used a training-test set analysis to compare different regression approaches seeking the optimum prediction.
- In Durban, South Africa, where the two dominant ethnic groups are Zulu and Indian, children presenting with nephrotic syndrome show a striking racial disparity: Indian children are roughly equally divided between steroid-sensitive and steroid-resistant nephrotic syndrome—the common pattern around the world—while Black (generally Zulu) children are almost always steroid-resistant. Seeking a genetic explanation, Dr. Rajendra Bhimma of the University of KwaZulu-Natal formed a collaboration with Dr. Cheryl Winkler. Dr. Winker's lab sequenced the NPHS2 gene, coding the essential kidney filter component, podocin, as mutations in this gene are the most frequent cause of childhood nephrotic syndrome. Thirty percent of the steroid-resistant nephrotic syndrome cases were found to result from homozygosity for a single mutation, V260E; this was highly unusual as this syndrome is usually found to be associated with many rare mutations, rather than a single mutation. The V260E mutation was previously reported from childhood nephrotic syndrome in several consanguineous families in regions around the Indian Ocean. We speculated that the unusual genetic pattern resulted from cryptic relatedness of our subjects, from introduction from Indian Ocean trade to a recent common ancestor—Durban is a port on the Indian Ocean. To test this hypothesis, we tested for homozygosity in the chromosome region surrounding NPHS2; a recent common ancestor for the two inherited copies would be revealed by an extend region of homozygosity. However, analysis of these stretches of homozygosity contradicted the idea of a recent common ancestor and showed rather that the likely time to the common ancestor was between 19 and 47 generations. This result—the age of the mutation in this population—is of great potential usefulness for precision medicine. Given the age, the mutation is likely to be present in a substantial Southern population of Zulu and related Bantu ethnic groups, and thus to be a frequent cause of childhood nephrotic syndrome in Southern

Africa. A simple and inexpensive genetic test could identify the underlying disease, saving many children from an unnecessary biopsy and a useless and dangerous course of steroid treatment.

With a responsive team that maintains expertise in the diverse domains of bioinformatics, and with close collaborations with both the CCR Sequencing Facility and various ABCC groups, the CCBR is an integral part of the CCR research community.

Office of Science and Technology Resources

The ABCC Scientific Web Programming Group (SWPG) maintains the build of the Office of Science and Technology Resources (OSTR) website (https://ostr.cancer.gov/) to support CCR and the OSTR. The project creates a single entity that consolidates all of the OSTR resources that are offered to NCI and customers of OSTR. The integration of the Supplemental Technology Award Review System (STARS) and Assay Depot (CREx) management modules (CREx) (https://nci.assaydepot.com) has also allowed a single data source to manage all of the services provided through OSTR. SWPG developed a tool that allows laboratories to be regularly notified regarding updates to their profiles and services offered to keep all information relevant, making the online marketplace a valuable resource to OSTR and the scientific community.

The laboratory information system (LIS) (https://lis.ncifcrf.gov/) continues to be utilized as a tool for scientific project submission and management at the laboratory level. This system acts as a stand-alone application as well as an interface for project submissions through the OSTR website's Sequencing Request Module. Continued development has further improved its functionality, allowing participants to organize resources and collaborate with one another through the life cycle of proposed projects. Actively it supports the Collaborative Protein Technology Resource, Optical Microscopy and Analysis Laboratory, and Sequencing Facility.

The ABCC also continued the management of the open-source Drupal CMS system and modules, including CREx.

Continued oversight for other OSTR-supported laboratories include: NCI Optical Microscopy Laboratories (https://confocal.cancer.gov), comprising the Laboratory of Cancer Biology and Genetics; Laboratory of Receptor Biology and Gene Expression; Cell and Cancer Biology Branch; Laboratory of Cellular and Molecular Biology; Experimental Immunology Branch; and Optical Microscopy and Analysis Laboratory. Support includes required Drupal management and updates as well as support of customer needs and requests.

Rockland and Abcam Antibody Request Portals

The ABCC SWPG has continued to support CCR antibody request portals for both Rockland Immunochemicals Inc., (https://ccrrockland.cancer.gov) and Abcam (https://ccr-abcam.cancer.gov). The projects were created to assist CCR in managing and reporting on custom requests for rabbit polyclonal and mouse monoclonal antibodies as well as to meet NIH standards and compliance requirements. These portals share a common code base and utilize the latest technology models. A single code base enables both sites to be easily managed while maintaining data integrity and privacy. Continued account support is offered as both portals continue to grow their user base and project lists.

CCR Office of Information Technology

The Patient Registration System (PRES) is a custom application that is designed to aggregate information from a number of data sources (as well as its own custom entry user interface) for the purposes of registering patients on new protocols. The progress to date has enabled users to quickly enter and manage patient data, and enhancements decreased page load times from 11 seconds down to 2 seconds, making the user experience much more efficient and less burdensome. Continued development includes adding features for users to streamline management of data as well as moving forward to implement the latest version of Labmatrix, adding greater security and flexibility to NCI and its customers.

Support Provided by the Clinical Monitoring Research Program

Leidos Biomed provides data management, medical support, and administrative services to the NCI Center for Cancer Research (CCR) and its clinical programs. CMRP provides comprehensive, dedicated clinical support to CCR, serving as an important resource for the development of new technologies and the translation of basic science discoveries into novel agents for the prevention, diagnosis, and treatment of cancer and AIDS. CMRP continues to propose new ideas to meet customer needs and modify existing positions, when possible, and to aid the continuing developments within each program. CMRP also provides programmatic and research subcontract support services to the various branches and sections within CCR. The support provided by Leidos Biomed and CMRP allow CCR to streamline operations, increase patient accrual, evaluate and treat patients more efficiently, and gather complete and accurate clinical data.

In support of CCR's protocol re-engineering, a CMRP clinical project manager manages a Basic Ordering Agreement (BOA) and Task Order (TO #3) awarded to Dilts + Partners, LLC, at the beginning of FY2016. The TO supports CCR's efforts to re-engineer its business processes related to the conduct of its research enterprise, including developing and opening clinical trials and effective managing of its research portfolio. NCI CCR envisions serving as a national model for cancer protocol development and review. In order to measure success, CCR seeks to continually establish, analyze, and adjust business metrics.

To date, Dilts + Partners has initiated work on the following: Vaccine Branch visioning packet, Urologic Oncology Branch visioning packet, the CCR communications campaign template, and CCR branding. In addition, Dilts + Partners completed the Lymphoid Malignancies Branch and Thoracic and Gastrointestinal Oncology Branch visioning and clinical resource planning meetings and held the Office of Science and Technology Resources (OSTR) kick-off meeting to begin development and planning of the OSTR Technology and Innovation Awards, created a framework for gathering and evaluating dimensions for OSTR performance, and planned for stakeholder input collection. Protocol re-engineering meetings continue, and Dilts + Partners continues to transition the clinical branch dashboards into the CCR-provided platform.

Office of the Director

CCR Office of Communications

CCR connections is a semiannual news magazine that launched in June 2007 and is available on the CCR's website, http://ccr.cancer.gov. The publication helps fulfill the CCR Office of the Director's mandate to deliver quality cancer information directly to the public as well as to health professionals within the oncology research community who, in turn, communicate with the public. This comprehensive resource, designed to meet the increasing demand for useful and authoritative information about important Intramural Research Program (IRP) advances, activities, and initiatives, provides the target audience with critical information about important research findings and the strategic partnerships that are producing innovations and inventions to better prevent, detect, diagnose, and treat cancer.

In July 2015, Leidos Biomed established a consulting agreement with Dr. Hemai Parthasarathy to produce two issues of *CCR connections* per year in support of the CCR Office of Communications. In the beginning of FY2016, CMRP staff exercised Option Period 1, extending the agreement through June 2016 and providing additional funds. Dr. Parthasarathy provides scientific writing and editing services for *CCR connections*, including content development, article and feature researching and writing, editing, and fact checking.

Dr. Parthasarathy completed work on two issues of *CCR connections*—one published in January 2016 and the other in July 2016. Both issues were published in print and online. These volumes consisted of feature articles, news articles, and patient stories, as well as other standard articles, such as "In the Clinic" and the "Director's Column." Due to a restructuring of functions in the CCR Office of the Director, Dr. Parthasarathy's agreement was not extended beyond June 2016. PMO staff managed this agreement through its conclusion at the end of June as well as activities required to close out the work effort.

Office of the Clinical Director

The NCI Office of the Clinical Director (OCD) serves as the interface between the CCR and the NIH Clinical Center. The NIH Clinical Center includes inpatient beds and day-hospital stations, which house units and clinics where cancer and HIV patients are treated during CCR clinical trials. The OCD oversees the quality of medical care delivered to patients participating in CCR clinical trials. The OCD also supports CCR's clinical research program by providing biostatistical expertise for trial design and analysis, administrative support for the protocol review and monitoring process, training of clinical research personnel, an outreach program to promote patient accrual, data management, auditing and monitoring of in-house and multi-institutional trials, and informatics for data collection and storage.

CMRP provides full-time shuttle and courier support services to and from the NIH campus and the OCD. The shuttle bus driver is responsible for transporting various staff members and time-sensitive documents (e.g., medical records) to the NIH campus.

A CMRP clinical program administrator supervises 13-plus staff members providing administrative support to various branches and sections within NCI/CCR. Additionally, the clinical program administrator serves as a liaison between other Leidos Biomed staff supporting NCI/CCR and the customer, and meets with team members and NCI/CCR leadership regularly to discuss ongoing work efforts and/or potential new work requests, employee relations issues, and teambuilding opportunities. Additionally, the clinical program administrator works with all NCI/CCR support staff on budget assumptions, manages budgets, and submits travel requests for program and HHS approval.

Within the reporting period, the clinical program administrator was instrumental in providing a detailed summary of all duties/activities performed by the PCC staff supporting various branches and sections within CCR. This summary allowed NCI/CCR division directors to review various teams' use of their PCC staff and examine potential opportunities for greater efficiency. This has become an invaluable tool in promoting better clinic flow for the patients and clinical teams.

The clinical program administrator organized and coordinated quarterly training sessions for the PCC staff that allowed them to broaden their knowledge base and enhance their contributions to their respective teams by incorporating various tools and learned skills to routine and new work assignments. The training sessions were given through CMRP's Learning and Professional Development (L&PD) group, keeping costs to a minimum.

The clinical program administrator continues to assist in developing and maintaining a communication plan for the NCI/CCR government customer and CMRP staff members and senior leadership in order to streamline policies and procedures when hiring Leidos Biomed staff.

The clinical program administrator also works closely with the NCI/CCR customer on Yellow Task (YT) requests and recruitment efforts. Over the past year, NCI/CCR increased requests for additional support staff. The clinical program administrator sent all new job postings to the government website TellCCR to increase visibility and open a larger pool of qualified candidates. The clinical program administrator also reviewed candidate applications, discussed candidates with hiring managers and teams, participated in interviews and meet-and-greet sessions, checked references, and prepared offer requests contingent upon favorable references.

IRB Administration Office

The Institutional Review Board (IRB) Administrative Office is under the direction of the CCR Office of the Clinical Director and provides administrative support for the NCI Intramural IRB. Currently, the IRB has approximately 400 protocols under its review. Staff reviews protocol actions for completeness before submission to the IRB chair, clinical director, and/or deputy clinical director for review and approval. Staff also writes minutes for the IRB and Safety Monitoring Committee (SMC) meetings.

The protocol coordinator assists IRB staff with the review of documents submitted by the principal investigators (PIs) and/or study coordinators and extracts relevant technical information to include in the IRB packets and database. The protocol coordinator is responsible for distributing correspondence and approved documents to the PIs and study contacts. Additional duties include processing and distributing other protocol actions (e.g., emergency-use Investigational New Drug [IND] forms, protocol status updates, short consent forms) using the iRIS database, assisting with IRB meeting packet preparation, monitoring the task box in iRIS, and routing issues to the appropriate analyst.

The protocol coordinator updates/maintains the CCR wiki for the IRB Administrative Office, prepares documents to renew IRB members before their terms expire, prepares the IRB meeting agendas for 23 annual meetings, and attends biweekly IRB meetings. The protocol coordinator also updates the standard operating procedures (SOPs) for Protocol Review Office (PRO) processes.

The medical writer attends IRB, Safety Monitoring Committee (SMC), and Scientific Review Committee (SRC) meetings; takes minutes; creates and edits SOPs and templates; and maintains the Protocol Support Office (PSO) wiki pages.

CMRP manages a research subcontract that provides the chair for the NCI IRB. The IRB chair position ensures the safety of clinical trial participants and the scientific validity of study findings. Support provided by the IRB chair includes: leading IRB meetings twice a month; playing an active role in establishing and reviewing IRB policies and procedures; identifying issues within research proposals; and conducting expedited reviews of recruitment materials, informed consents, and special exemptions. Due to a new study application, PRO staff assisted CCR employees with a step-by-step process on how to complete the application.

CMRP staff has taken on additional work efforts in FY2016, serving as the backup for PRO staff and assisting with iRIS issues that may occur with the Ethics Office. The protocol coordinator completes the full board and expedited continuing reviews, while still completing amendments.

The CMRP medical writer was previously part of the PSO, but the position moved to this office in October 2015. The medical writer attends 23 IRB meetings and four SMC meetings annually and writes minutes for each. In addition, the medical writer creates templates, writes SOPs, manages wiki pages, and manages meeting space.

Protocol Support Office

The CCR re-engineered its processes related to the development, review, and initiation of clinical trials in order to decrease the time from scientific review to opening clinical trials for patient accrual, while maintaining or increasing quality and safety. As a result, CCR established the PSO, in addition to the IRB Administration Office. The PSO oversees services in three major areas: (1) writing and editing, (2) regulatory and compliance, and (3) protocol navigation and administration.

In support of the PSO, CMRP employs four protocol coordinators and two medical writers. The protocol coordinators serve as liaisons between CMRP and CCR/NCI staff to initiate and complete tasks related to protocol support. The two medical writers attend IRB, Safety Monitoring Committee (SMC), and SRC meetings; take minutes; create and edit SOPs and templates; maintain the PSO wiki pages; and review and edit protocol amendments and continuing reviews.

CMRP is responsible for assisting with the preparation of new proposals/protocols and progress reports for IRB meetings and assisting PSO staff in reviewing and making recommendations and/or changes to protocol amendments and other documents related to research studies. The team also assists with training new staff. Members of the PSO are required to attend IRB, SMC, and SRC meetings. CMRP staff administers, organizes, and coordinates the CCR SRC meetings and supports investigators with preparing submissions under the new process. In addition, staff members are in charge of coordinating branch and concept reviews, and contacting PIs for the review boards as needed.

CMRP staff assists with the preparation of new proposals/protocols and progress reports for IRB meetings. CMRP staff also assists PSO staff in reviewing and making recommendations and/or changes to protocol amendments and other documents related to research studies. Members of the PSO are required to attend IRB, SMC, and SRC meetings. In addition, staff members are in charge of contacting PIs for the review board as needed.

Staff administers, organizes, and coordinates the CCR Scientific Review meetings, and supports investigators with preparing submissions under the new process.

CMRP staff members have played key roles in processing clinical protocols for submission to various regulatory agencies, such as the IRB, the Food and Drug Administration (FDA), the Office of Biotechnology Activities (OBA), and the Institutional Biosafety Committee (IBC). The team assisted clinical investigators with the review of 31 new protocols and informed consent documents, 72 protocol amendments, and 32 OBA/IBC submissions. Staff was involved in 42 protocol navigation projects and three NIH Office of Human Subject Recruitment and Protection (OHSRP) applications, as well as in the maintenance of 40 regulatory binders. Currently, the team provides support to 18 different branches within CCR for approximately 19 active Investigational New Drugs/Investigational Device Exemptions/Drug Master Files.

Staff members were involved with the National Marrow Donor Program (NMDP) in a memorandum of understanding agreement between OHSRP and NMDP. The agreement stated that, in order to maintain donor confidentiality, unrelated donor source documents will not be sent to NIH.

Endocrine Oncology Branch

The Endocrine Oncology Branch (EOB) focuses on treating patients with endocrine malignancies. The ultimate goal of the EOB is to establish an integrated basic, translational, and clinical research program with the goal of developing innovative diagnostic and prognostic approaches and treatments for endocrine cancers. This goal is consistent with the mission of the CCR to understand the causes and mechanisms of cancer, improve early detection and diagnosis of cancer, understand the factors that influence cancer outcomes, and develop effective and efficient treatments for patients with cancer.

EOB is studying types of thyroid cancer that seem to cluster in families. Patients are evaluated, and the study team looks for genes that may cause the cancer, the goal being to develop the best ways to screen for this disease so that it can be diagnosed and treated at an early stage. This research is intended to help better understand how cancer develops in persons from families where thyroid cancer is prevalent; eventually, the results of this research might help other people with cancer or who have a genetic risk of developing cancer in the future. Participants come from across the world to take part in the genetic mutation DNA sequence study for familial non-medullary thyroid carcinoma.

For the EOB's newest treatment study (15-C-0040, Targeted Therapy in Advanced Neuroendocrine Tumors), patients will receive treatment with either Sunitinib or Everolimus based on the germline or somatic mutations identified by the tumor genotyping. The program is also implementing three more treatment studies that CMRP staff will help support by facilitating patient recruitment and ensuring coordination of care.

Experimental Transplant and Immunology Branch

The Experimental Transplant and Immunology Branch (ETIB) is dedicated to coordinating efforts for basic, preclinical, and clinical investigations related to transplantation science. The goal of this program is to generate information from basic and preclinical investigations that leads to the development of novel, curative therapies for cancer. Information from new treatment protocols, including novel endpoints generated in the course of basic and preclinical research, is used to generate questions and studies in basic and preclinical research efforts.

ETIB currently has 25 open protocols covering research related to transplants, cell therapy, natural history, specimen collection, and treatment protocols, as well as one that supports the outcomes data collection federal mandate to report data to the Center for International Blood and Marrow Transplant Research (CIBMTR).

CMRP provides clinical professionals to support ETIB (i.e., licensed independent practitioners, PCCs, a clinical research nurse, and a clinical nurse administrator). CMRP staff facilitates clinic-related functions, coordinates administrative support and patient recruitment, processes patient intakes, facilitates patient accrual, and maintains communication with patients and referring physicians' offices. Bilingual CMRP staff members serve as a point of contact for the branch's many Spanish-speaking patients and assist with the development of Spanish educational materials.

Three CMRP physician extenders provide clinical care to patients with complex, multisystem problems in the inpatient and outpatient settings. They support and educate patients, family members, and staff, including NCI, NHLBI, and Georgetown University Hospital fellows on matters relating to transplant, health and research studies, and specific ETIB protocols. The physician extenders participate in clinical rounds and meetings/conferences related to study protocols and research. Two physician extenders provide nighttime coverage for ETIB and NCI Medical Oncology Branch patients, a difficult position to fill with permanent staff. Consistent, long-term nighttime care positively impacts the continuity of care for patients who are in the ETIB system for months to years.

The CMRP clinical nurse administrator supports the ETIB studies that utilize unrelated donor grafts and also manages the overall program needs for the cross-institute NIH Unrelated Donor Hematopoietic Stem Cell Transplant Program. The clinical nurse administrator provides donor search and graft procurement service; during FY2016, approximately 180 preliminary searches and 50 formal searches were performed, and approximately 30 grafts were procured. The clinical nurse administrator facilitates NIH transplant protocol submission to the National Marrow Donor Program (NMDP) IRB; advises on donor and cord blood selection; coordinates the donor search and procurement of marrow, peripheral blood stem cells, and cord blood from the U.S.

and international donor and cord blood centers; ensures compliance with applicable guidelines, Transplant Center recertification, budgeting, planning, and outcomes reporting; and provides regular lectures to rotating NCI/NHLBI/Georgetown fellows.

A new CMRP PCC was added to support the transplant coordinator's office. This role includes reviewing and organizing documents/records, coordinating recipient and donor human leukocyte antigen (HLA) kits, and assisting with scheduling patient appointments to maximize the efficiency of the office, allowing potential protocol participants to be screened more accurately and quickly.

The clinical research nurse aided in the development and implementation of two new protocols and managed a complicated communication system between NIH and Bluebird, an external company involved with one of the protocols. The clinical research nurse also completed a continuing review and two audits in this period, updated processes and procedures to comply with regulatory requirements, and assisted with amendments to existing NHLBI protocols.

The cost of unrelated donor grafts is typically covered by private health insurers through hospital contracts. In the government setting, this cost is borne by NIH, and cost savings on graft search and procurement directly benefits the patients by allowing for additional searches and graft procurements. The new agreement established with the New York Blood Center (NYBC) during the reporting period will allow both unlicensed and licensed cord blood units to be procured directly from NYBC rather than procuring the units through the National NMDP, resulting in a cost savings of approximately $6,450 per unit purchased. Additionally, due to the ability to purchase units directly, the cost of confirmatory typing cord blood units through the NYBC will be zero. This will save as much as $1,400 per unit typed; NIH typically types one to two units per patient. CMRP staff arranged internal courier service for stem cell grafts that are collected within a two-hour drive from Bethesda, MD, saving $10,025.

Reporting of transplant outcomes is federally mandated by the Stem Cell Therapeutic and Research Act of 2005, which authorized the C.W. Bill Young Cell Transplantation Program. The organization charged with collecting this data is the CIBMTR. During a 2015 audit, several areas of concern were brought to NIH's attention. This involved data from all reporting centers, including NIAID, NCI, and NHLBI. CMRP staff are involved in several new initiatives to improve the accuracy of documentation, both pre- and post-transplantation. A team was developed to track clinical notes that must be documented at specific time points. The CMRP PCC created a calendar and reminder system and worked with physician extenders to ensure timely and accurate completion of these notes. Infusion data was also identified as a source of frequent errors. CMRP staff is part of a small group working with NCI, NHLBI, NIAID, Harris, Inc. (who has the data management contract for all three institutes), and the Department of Transfusion Medicine (DTM) staff to develop a long-term solution to improve cell therapy documentation in medical records. As a short-term solution, CMRP staff assisted with the development of infusion form completion guidelines that will be used to create a data management SOP.

The CMRP clinical nurse administrator coordinated the first unrelated donor graft on a long-standing NHLBI protocol that requires donors to undergo unstimulated lymphocyte collection prior to the stem cell donation. This was the first collection of its kind for NMDP, and the lymphocyte collection took place three weeks after the high-resolution typing of the recipient was complete (typical turnaround is six to eight weeks). This one collection necessitated interagency funding transfers, an NMDP NIH Reliance Agreement, additional testing and evaluation for Zika and malaria risk, efforts in DTM to perform HLA testing within days instead of weeks, and balancing the interests of the donor and recipient by maintaining direct contact with the apheresis center (uncommon) during a complicated collection.

In late 2015, a cell administration event led to an Occurrence Reporting System (ORS), which, in turn, led to the creation of the Clinical Center-led Failure Modes and Effects Analysis (FMEA) Team. CMRP clinical staff attended these meetings to provide input on potential failure modes, hazard risk, and potential solutions. As a result of these preliminary meetings, two subgroups were formed to address delays in the administration of cell therapy and documentation of adverse events during infusion. A new Clinical Research Information System (CRIS) service request and associated clinical note are in development to address the lack of documentation of a request for an unrelated stem cell donor search, and the results and analysis. This is particularly relevant with the introduction of several new transplant protocols utilizing haploidentical family members, as the eligibility criteria and standard practice is to document the lack of an available matched unrelated donor prior to making the decision to use a less well-matched donor. The current system of emails and unsearchable nursing notes does not adequately address this need. A template is being developed to address consistency and functionality of these notes, and the process will be presented to institute users (physicians, research nurses, and research coordinators at NCI, NHLBI, and NIAID) and at the Blood and Marrow Transplant (BMT) Consortium for review in FY2016.

CMRP staff members expertly manage the conflicting needs and priorities of multiple NIH intramural institute programs and develop trust by conducting a fair and equitable distribution of available unrelated donor grafts, communicating with program leadership when additional funding is required to meet patient needs, and managing a complex budget with funds in four separate projects. The multi-faceted, cross-institute Unrelated Donor Program requires superior skills in communication. The CMRP nurse administrator prioritizes customer service skills by building effective working relationships with outside

stakeholders, coordinating program activities and protocol developments, and collaborating with multiple institutes/ sections within and outside of NIH. She participates in clinical team meetings, the Cross Institute BMT Consortium, Clinical Center workgroups, and quality control groups dedicated to improving patient safety for transplant recipients regardless of graft source, protocol, or institute.

CMRP staff members continue to work across many departments, branches, and institutes to support research objectives, patient safety, and facilitate cross-institute referrals and communications. The physician extenders and clinical nurse administrator provide Fellows Lectures to other institutes and to outside staff rotating on ETIB service, and are sought out to provide advice on donor and cord blood selection for other hospitals.

Subcontract and Blanket/Purchase Order Support

CMRP continued to provide oversight and management of the agreement with NMDP that allows the NMDP-maintained database of donors to be searched for potential matches and allows for donor testing, graft products, and other related services to be obtained for participants enrolled in NIH protocols that require a matched unrelated donor product. A new agreement with the NYBC was negotiated and established in FY2016 to allow licensed (HEMACORD®) and unlicensed cord blood units to be procured through the NYBC National Cord Blood Program (NCBP) for study participants. In addition, Bone Marrow Donors Worldwide (BMDW) is a database service that is utilized to search for donor stem cell and cryopreserved cord blood units in order to identify potential matches for study participants. Until recently, the BMDW database was a free search service, but it now requires a yearly user fee. CMRP established BMDW as a new vendor to process the user fee and allow continued availability of search services to the clinical staff.

HIV and AIDS Malignancy Branch

The HIV and AIDS Malignancy Branch (HAMB) conducts laboratory and clinical research on HIV disease, AIDS-related malignancies, viral-induced tumors, and related diseases. HAMB investigators also dedicate research efforts to observing the pathogenesis of Kaposi's sarcoma-associated herpesvirus (KSHV).

A CMRP PCC provides administrative support to the HAMB team, which consists of three investigators and two research nurses; approximately 275 patients are enrolled on 10 active protocols. The PCC created a shared calendar for the team to view patient treatment cycle dates, as well as associated times for staff to complete outside pharmacy drug forms for the drug sponsor to send the study drug to the NIH pharmacy prior to patients' appointments.

The HAMB team, with the support of the PCC, received approval for two new protocols to continue the treatment of KSHV and expand HAMB's clinical research focus by treating other malignancies (e.g., anal, stomach, and esophageal cancer) with similar approved therapies used in treating KSHV.

The HAMB team and PCC are pursuing the development of two additional protocols that are pending approval by the SRC before proceeding to the IRB process. Additionally, the PCC developed a tracking system to capture which patients required re-consenting to an amended version of a protocol, based on a language change in a previously signed version of the informed consent document.

Lymphoid Malignancies Branch

The Lymphoid Malignancies Branch (LYMB) focuses on the identification of abnormalities in the regulation of the immune response and the definition of molecular disorders that underlie lymphoid malignancies. A mainstay of LYMB is gene-expression profiling, which provides a foundation for understanding the molecular pathogenesis of these cancers and their response to therapies. A research goal is to define the pivotal roles played by IL-2 and IL-15 in the life and death of normal and neoplastic lymphocytes. The branch also focuses on T-cell malignancies, with emphasis on human T-cell lymphotropic virus 1, which is associated with adult T-cell leukemia. The major clinical research emphasis is to develop the most effective treatment in molecular subtypes of aggressive B-cell lymphomas.

During this reporting period, LYMB launched two new protocols and maintained 15 active protocols. One of the new protocols (# 14-C-0157) is a Phase I study focusing on primary central nervous system lymphoma (a rare form of diffuse large B-cell lymphoma), which is difficult to treat around the blood-brain barrier.

CMRP implemented a Medical Oncology Branch (MOB) Referral Office, comprising clinical research nurses that interview patients prior to referring them to the specific teams. The service allows PCCs and nurses to focus on patient and program needs internally while gaining assistance from the referral office for external relations, such as requests for outside patient records and pathology slides.

A CMRP PCC provides administrative support to LYMB's clinical research efforts. During this reporting period, the PCC was assigned additional duties in regards to clinic scheduling and communication among teams within LYMB. The PCC facilitates all administrative activities and coordinates schedules for new and currently enrolled LYMB patients.

Pediatric Oncology Branch

CCR's Pediatric Oncology Branch (POB) provides support to numerous multifacility studies researching the effects of chronic illnesses, brain tumors, and experimental drugs on pediatric and adult patients. The main objectives are to study the effects of disease and treatment on the functioning of children and adults with chronic illness through comprehensive longitudinal assessments, neuropsychological measurements, neurological abnormalities, advanced imaging, laboratory testing, morbidity assessments, and psychological factors.

This results in a comprehensive overview of all baseline deficits, declines, or improvements related to treatment and the disease.

CMRP PCCs support IRB-approved protocols by assisting with patient recruitment, creating new patient information packets, educating patients and their families about pediatric protocol processes, tracking patient assessments, and helping to ensure that patients are not lost to follow-up. Spreadsheets and databases are used, allowing efficient ways to combine evaluations from different studies and track patient information.

The POB opened protocol 15-C-0168, treating brain tumors with Polmalidamide in September 2015 and is currently two patients away from completing accrual.

In an effort to save on the cost of paper, POB coordinated the set-up of an electronic group fax to help streamline offsite data collection and outside medical records. Records no longer need to be printed and sent to medical records for electronic scanning; they are now directly sent to each team member's email for review. The POB has also created a shared patient calendar through Outlook. Team members can now access all scheduling dates and appointments individually, at their convenience, without needing a paper copy.

Behavioral Health Care

POB includes a Behavioral Health Core that consists of two separate, interrelated components: the neuro-behavioral program and the psychosocial program. The Behavioral Health Core was created to facilitate the development of studies investigating the neuropsycho-logical and psychosocial effects of chronic illness, provide specialized research support to clinical trials using neuropsychological and quality-of-life outcome measurements, and offer clinical services to the patients and families enrolled in studies throughout NCI.

The main objectives of the neurobehavioral program are to study the effects of disease and treatment on the neurobehavioral functioning of children and adults with chronic illness through comprehensive, state-of-the-art longitudinal assessments; examine the pathogenesis of central nervous system dysfunction by exploring the relationships of neuropsychological measurements to disease parameters, neurological abnormalities, biomedical and genetic variables, and environmental and psychological factors; develop new measures to identify the psychosocial and neurobehavioral effects of cancer and other chronic illness; and investigate potential cognitive interventions that could help ameliorate some of the cognitive deficits and declines as a result of the disease and treatment.

The neurobehavioral program also offers clinical services to patients, including: providing assessment results to families, making recommendations and coordinating at-home psychoeducational services, and implementing clinical interventions based on patient needs. Additionally, the program conducts training that provides psychology students with valuable clinical and research experience in a medical setting.

The CMRP psychometrician works primarily with the neurobehavioral program to conduct longitudinal neurobehavioral assessments of children, adolescents, and adults enrolled in collaborative research protocols or in response to clinical referrals. The psychometrician is also responsible for ensuring that test data is coded accurately and entered into the system routinely so the customer can perform its analyses when requested by PIs of some of the protocols supported by the neurobehavioral program.

During FY2016, the psychometrician was part of the team that supported 16 IRB-approved protocols. The psychometrician conducted 45 comprehensive protocol-related assessments during this period; in addition, five hours of clinical intervention (therapy) was provided as a service to patients referred due to social-emotional issues. The psychometrician also completed two clinical reports as requested by the families. These reports summarized the results from the psychological testing and contained recommendations for use in an educational setting.

Three new protocols were launched by the Behavioral Health Core in 2015: 15-C-0019, The Impact of the RAS/MAPK Signaling Pathway-Targeted Therapies on Neurocognitive Functioning in Individuals with Neurofibromatosis Type 1 (NF1); 15-C-0142, Acceptance and Commitment Training for Adolescents and Young Adults with NF1, Plexiform Neurofibromas (PNs), and Chronic Pain: A Phase III Clinical Trial; and 15-C-0195, Development and Validation of Patient Reported Outcome (PRO) Measures for Individuals with NF1 and PNs.

Protocol 15-C-0019 is a collaboration with the Division of Pediatric Neuropsychology, Children's National Medical Center. The protocol has enrolled 35 patients, and more than half of these patients are new patient recruits. For this protocol, the psychometrician administers computerized and hard copy evaluations to assess specific areas of functioning: attention, processing speed, working memory, and mental flexibility.

Protocol 15-C-0142 has enrolled 27 patients since it opened for enrollment in June 2015. For this protocol, the psychometrician administers pain and quality of life measurements and then conducts one-on-one training with patients to learn the basic concepts of the third-generation cognitive therapy called Acceptance and Commitment Therapy. In this training, the psychometrician teaches patients specific skills that could help them better cope with pain and live a valued life.

Protocol 15-C-0195 is a multicenter study that seeks to modify existing instruments of pain intensity and interference for use as endpoints in clinical trials with NF1 and PNs. Secondary objectives include developing normative data and establishing reliability, validity, sensitivity to change, and feasibility of measures with individuals with NF1 and PNs. For this protocol, the psychometrician assists the principal and associate investigators in conducting focus groups or individual interviews with the target population to get feedback about pain intensity and interference measures commonly used in research. The goal is to see if participants felt that the measures were relevant to them and their experience of pain.

Radiation Oncology Branch

The Radiation Oncology Branch (ROB) designs and conducts preclinical and clinical research on the biologic and therapeutic effects of radiation therapy. The clinical trials that ROB develops and conducts involve novel technology and/or imaging-based approaches to radiation therapy treatment.

CMRP currently has two PCCs and one clinical program administrator supporting ROB. These individuals schedule patient appointments, create clinic schedules, manage patients' electronic medical records, and refer phone calls. The clinical program administrator maintains office supplies and manages the daily operations within ROB.

During the last reporting period, the CMRP clinical support staff provided protocol support to the Neuro-Oncology Branch (NOB) to assist during the absence of a branch chief. ROB no longer follows NOB's patients (approximately 100) and has relinquished most related support. Also since the last reporting period, the ROB nursing staff levels have returned to normal; however, CMRP PCCs have continued to provide multidisciplinary coverage, which has included disseminating patient welcome packages, entering admissions/travel/voucher information, sending scheduling notices, requesting outside records, obtaining screening consent, and scheduling patient follow-up visits. The CMRP PCCs completed training on the ARIA® oncology information system. Now that it is fully implemented, ARIA® has allowed the CMRP PCC staff to upload various medical records and manage the patient's entire journey from initial diagnosis through post-treatment follow-up.

Thoracic and Gastrointestinal Oncology Branch

The main objective of the Thoracic and Gastrointestinal Oncology Branch (TGIB) is to conduct laboratory and clinical research focused on improving the care, management, and outcomes of patients by developing innovative surgical and adjunctive approaches.

The TGIB support team consists of two clinical research associates (CRAs) and one clinical coordinator. These staff members support six clinical investigators (three within the thoracic team and three within the GI team), who conduct 10 active clinical studies and have been instrumental in maintaining the work for the TGIB. Data support is provided by the CRAs, and patient care coordination is provided by the clinical coordinator. The staff works closely with the PIs and research nurses to ensure all support activities are handled in a timely and efficient manner.

Both CRAs support five protocols that have accrued approximately 418 patients. They also assist with data and statistical analyses. Additionally, both CRAs train surgical fellows on a variety of surgical oncology projects, including toxicity assessments, review of data, and patient records.

The CRAs assist CCR PSO staff with reports for NCI IRB continuing reviews, assist surgical fellows and PIs in creating and completing database forms for use in the clinic, and assist in evaluating and following research-related adverse events and toxicity assessments for patients participating in active clinical trials.

The CRAs support the GI team by providing assistance with data and logistics management for consulting services provided by the GI team PIs for 433 consult patients. Of the 433 consult patients, 262 patients were scheduled and had undergone surgeries performed by GI team PIs.

The CRAs continue to maintain the surgical caseload spreadsheet for the surgeries performed by the GI team PIs. This spreadsheet has proven to be important in avoiding scheduling conflicts and providing a record of each PI's surgical caseload.

The CRAs reviewed and contributed data for a company-sponsored percutaneous hepatic perfusion Phase III multicenter trial. The CMRP support team served as the clinical coordinating center and provided data analysis support for this large and complicated study. The final results were published in the *Ann Surg Oncol*.

The CRAs started preliminary preparation to support new protocols being developed under the new Gastric Trials program. This program will include at least three new protocols for gastric cancer treatment. These protocols are expected to be open by the end of this calendar year and are in early stages of protocol development. The CRAs are assisting with protocol review, creation of electronic regulatory files, enrollment logs, study templates, entry and eligibility criteria, and collection of educational information for prospective patients.

The clinical coordinator provides patient care coordination support to three investigators for five protocols for the thoracic team, scheduling patient appointments, testing, screenings, consults, itineraries, and lodging.

The clinical coordinator continues to maintain the Outlook calendar for the thoracic team, adding patient schedules, surgery dates, admissions, and pre-operative schedules to the calendar to ensure everyone has current scheduling information. The clinical coordinator continues to assist the thoracic team research nurse with vaccine product delivery for one of the team's studies from the laboratory to the patient clinic (14-C-0053, Adjuvant Tumor Lysate Vaccine and Iscomatrix with or without Metronomic Oral Cyclophosphamide and Celecoxib in Patients with Malignancies Involving Lungs, Esophagus, Pleura, or Mediastinum).

One new protocol (Phase I Evaluation of Oral Decitabine and Tetrahydrouridine with or without Celecoxib in Patients Undergoing Pulmonary Metastasectomy) that went through internal review during the last reporting period will be submitted to the IRB and the Food and Drug Administration (FDA) as a new protocol/Investigational New Drug (IND) once the manufacturing issues raised by the shut-down of the NIH Pharmaceutical Development Section have been resolved.

Two new thoracic team protocols have completed internal scientific review and will be submitted to the IRB and the FDA as new INDs: (1) Phase I/II Evaluation of Continuous 24h Intravenous Infusion of Mithramycin, an Inhibitor of Cancer Stem Cell Signaling, in Patients with Primary Thoracic Malignancies or Carcinomas, Sarcomas or Germ Cell Neoplasms with Pleuropulmonary Metastases; and (2) Phase I/II Evaluation of Oral Decitabine and Tetrahydrouridine with Nivolumab, an Immune Checkpoint Inhibitor, as First-Line Therapy for Inoperable Non-Small Cell Lung Cancer.

Surgery Branch (Immunotherapy)

The Surgery Branch (SB) is a combined laboratory and clinical research unit devoted to the development of innovative cancer immunotherapies and their translation to the treatment of patients with cancer. Efforts focus on improving the care, management, and outcomes of patients by developing innovative, autologous T-cell/gene therapy, and surgical and adjunctive approaches. The SB's pioneering immunotherapy research has been featured in news articles and video clips; a Discovery Channel documentary on Building 10/NIH Clinical Center is in production that will include a segment on the SB.

CMRP supports the SB with a senior IND manager, a protocol development coordinator, and a bioinformatics analyst to provide regulatory, administrative, and scientific support for the SB PSO and SB laboratories.

The senior IND manager assists other SB PSO staff with submission and maintenance of protocols; reviews, updates, and writes SOPs; creates template documents for routine submissions to the SB master file and FDA; processes continuing reviews; and prepares IND annual reports and IND applications.

The protocol development coordinator provides an organizational framework for tracking due dates to ensure processing and timely submissions of protocols, maintains databases to track all submissions to various regulatory agencies, and maintains a database of SB patient screenings, referrals, and regulatory submissions.

The bioinformatics analyst supports the principal investigators with enrichment analysis, statistical analysis of the genomic sequencing, and single-cell microarray analysis. Mouse genome data analysis to determine the mutations, amplification and deletion regions, gene expression, and phylogeny is conducted using fresh tumor samples and tumor cell lines. New work has also been initiated to generate microarray datasets to compare with the current T-cell receptor (TCR) dataset from the National Center for Biotechnology Information Gene Expression Omnibus database (NCBI GEO); to assist with methylation-sequencing analysis to determine the significant methylated regions of the sequence in order to find methylated mutations; and to conduct copy number analysis, correct for copy number calls, and develop a pipeline for clonality analysis.

To better support SB research, the bioinformatics analyst has made several suggestions to improve efficiencies. These include: submitting normal mouse samples for sequencing to compare and verify expression of tumor mutations, recommending more efficient ways to determine differences between two groups from the ribonucleic acid-sequencing (RNA-seq) data without enriched gene sets by using hierarchical cluster and pathway analysis, and enhancing multiple mutation detection techniques by adding four different methods to help reduce false positives and increase accuracy.

During FY2016, three new protocols were submitted to CCR for initial concept review. Of these, two protocols (one Phase II, one Phase I/II) are going to be new INDs sponsored by SB investigators and may be submitted to the NCI IRB, IBC, Office of Scientific Programs (OSP), and FDA. The timeline for protocol submission is dependent upon renovations and restructuring of the SB Cell and Vector Production facilities. The third protocol is a company-sponsored, multicenter Phase I/II study for which SB is one of the sites.

A protocol submitted to FDA as a new IND received NCI IRB approval (Phase I/II Study Administering Peripheral Blood Lymphocytes Transduced with a CD70-binding chimeric T-Cell Receptor to Patients with CD70 Expressing Cancers), and another protocol that was submitted to and approved by the NCI IRB and FDA under an existing IND is currently enrolling patients (A Prospective Randomized and Phase II Trial for Metastatic Melanoma Using Adoptive Cell Therapy with Tumor Infiltrating Lymphocytes Plus IL-2 Either Alone or Following the Administration of Pembrolizumab).

Due to an FDA "for cause" audit of the Pharmaceutical Development Section (PDS) at the NIH Clinical Center in May 2015, the NIH leadership and CCR retained external consultants to proactively conduct internal reviews of all NIH cell production/manufacturing facilities. As a result of this voluntary audit's findings, NIH audited additional facilities, including the vector labs. INDs for the SB and vector lab facilities are currently on administrative hold, which has presented challenges for active SB protocols and the SB cell and vector production facilities, which are currently undergoing renovations to meet Good Manufacturing Practice (GMP) standards. The SB administrative team has worked hard to mitigate issues, respond to information requests, and continue to provide protocol/regulatory support.

Changes are underway to transition the SB PSO staff to the central CCR Office of Regulatory Affairs, which will also include transferring sponsorship of the SB protocols and INDs to CCR; several change-in-sponsor/acceptance-of-sponsor IND submissions have been processed for submission to FDA and protocol amendments are being submitted to the NCI IRB. Once the transfer of all protocols and INDs is completed, the portfolio assignment for SB PSO staff under the CCR Office of Regulatory Affairs will be updated.

Urologic Oncology Branch

The Urologic Oncology Branch (UOB) conducts clinical and bench research focused on developing new treatments, detection, and prevention methods for genitourinary malignancies, including bladder, kidney, and prostate cancers. The bladder team focuses on developing new and/or improved treatment for non-muscle invasive bladder cancer. The kidney team focuses on understanding the genes associated with the occurrence and progression of kidney cancers.

CMRP staff members supporting UOB actively participate in all aspects of the UOB's research program, including patient recruitment and scheduling, clinical trial enrollment, clinical evaluation and management of patients undergoing treatment, continuity of care, patient education, and medical team consultation coordination.

The bladder team has continued to provide care for complex urothelial cell carcinoma patients, as well as standard of care surgery (e.g., biopsies, tumor resections, and cystectomies) and intravesical bladder treatment (BCG, gemcitabine) for tissue procurement protocols (97-C-0147; 15-C-0087). Additionally, the team has continued to enroll patients on the Phase II trial for patients with BCG-refractory high-grade, non-muscle invasive bladder cancer that evaluates the combination of BCG with a novel vaccine developed at the NIH called PANVAC (14-C-0036). Protocol 15-C-0087 began patient accrual in November 2015 and the bladder team is working to open this protocol at other local centers, including Washington Hospital Center and Georgetown. The UOB bladder team continued to enroll patients in the Phase II trial for patients with BCG-refractory, high-grade, non-muscle invasive bladder cancer (that evaluates the combination of BCG with a novel vaccine developed at the NIH called PANVAC), both at NCI and the secondary study site at Rutgers Cancer Institute of New Jersey. Additional treatment protocols are expected to open in FY2016.

The prostate team research efforts remain focused on: investigation of multiparametric MRI (mpMRI) in prostate cancer to improve diagnosis with mpMRI whole-mount pathology correlation and creation of a tumor-directed biopsy device; development of image-guided focal prostate cancer therapy; and evaluation of immunotherapy, molecular targeted therapy or other pharmacotherapies in management of localized or locally advanced prostate cancer. Currently, the team has three active protocols. This fiscal year, protocol 16-C-0010 opened to accrual; it is expected to accrue 3,000 prostate cancer patients to provide care and prospective procurement of prostate cancer cells. Furthermore, ongoing studies include: Protocol 14-C-0112, a Phase II study of neoadjuvant rFowlpox-PSA (L155) TRICOM in combination with rVaccina-PSA (L155) TICOM in men undergoing prostate cancer treatment with radical prostatectomy, and Protocol 15C0205, a Phase II randomized, placebo-controlled study of PROSTVAC in patients with clinically localized prostate cancer undergoing active surveillance. The staff provides support to the 130-plus participants currently enrolled.

Additionally, in FY2016, the bladder team promoted communication and collaboration among the team and colleagues in medical and radiation oncology. The team established a weekly multidisciplinary bladder cancer meeting to discuss mutual patients; M.D.s, NP/Pas, and RNs from each team, and representatives from radiology and pathology attend these meetings to review patient imaging and biopsy results, and to discuss treatment planning.

Laboratory of Molecular Biology

The Laboratory of Molecular Biology (LMB) focuses on three major areas of research to understand and treat cancer: the study of antibody-based cancer treatments for hematologic-, liver-, and mesothelin-based cancers; the modeling of thyroid hormone-based cancers; and the basic biochemical processes of gene transcription, post-translational regulation, molecular chaperone systems that manage protein damage, and the localization and assembly of large protein structures. A CMRP senior nurse practitioner supports the PI conducting clinical trials of recombinant immunotoxins for hairy cell leukemia, a chronic B-cell lymphoproliferative disorder, as well as other hematologic malignancies such as adult T-cell leukemia/lymphoma, a rare, aggressive, and deadly disease. Current research protocols focus on using varying regimens of treatment with purine analog in combination with monoclonal antibody for newly diagnosed or once-treated hairy cell leukemia patients. In addition, LMB has several protocols directed at relapsed hairy cell leukemia with a variety of oral agents, chemotherapy with monoclonal antibody, and a novel, targeted immunotoxin therapy that has proven highly successful in eliminating minimal residual disease for long-term complete remission.

The nurse practitioner position serves in a leadership role as an associate investigator for all treatment-related protocols. She supports Dr. Robert Kreitman and a team of three research nurses by reviewing new patient records and writing concise histories of present illness by interviewing patients and outlining pertinent co-morbidities and medication lists. In conjunction with Dr. Kreitman, the nurse practitioner manages patients who are in active treatment by performing initial history and physical exams, evaluating lab and imaging results, and determining if patients can safely proceed with treatment at specified time-points. The nurse practitioner also evaluates and manages co-morbid conditions, treatment and disease-related symptoms/toxicities such as nausea, rash, fatigue, etc., throughout the active treatment cycle, and sees patients for routine protocol-driven follow-up evaluations after treatment completion.

The currently active treatment protocols are as follows:

1. Protocol 09-C-0005: "A Randomized Trial of Cladribine with Simultaneous or Delayed Rituximab to Eliminate Hairy Cell Leukemia Minimal Residual Disease." This protocol is available to newly diagnosed hairy cell leukemia patients who meet

specified eligibility criteria. Following treatment, all patients are closely monitored with quarterly lab tests for the first two-and-a-half years and then biannually after that. There are currently 137 patients who are actively being followed on this protocol. The treatment is highly effective, so patients are seen in long-term follow-up for years after treatment.

2. Protocol 09-C-0025: "A Phase II trial of LMB-1, fludarabine and cyclophosphamide (FC) for adult T-cell leukemia." Adult T-cell leukemia (ATL) is a rare, highly fatal, and aggressive disease. The protocol's primary objective is to determine, in nonrandomized fashion, if, after verifying its safety, fludarabine and cyclophosphamide prior to LMB2 for ATL can result in low immunogenicity and a rate of major response lasting greater than eight weeks, which may be an improvement over that demonstrated previously from CAMPATH. Secondary objectives include determining the effect of one cycle of FC alone in ATL and examining progression-free and overall survival in ATL after FC/LMB-2. This study also evaluates the pharmacokinetics, toxicity, and monitors soluble CD25 and other tumor marker levels in the serum. A total of 31 patients have been treated with this protocol, and one is currently in long-term follow-up.
3. Protocol 10-C-0025: "A Randomized Phase II Trial of Rituximab with Either Pentostatin or Bendamustine for Multiply Relapsed or Refractory Hairy Cell Leukemia." The primary objective of this study is to determine which regimen is superior in treating refractory disease, as well as to determine if minimal residual disease levels and tumor markers (soluble CD25 and CD22) correlate with response and clinical endpoints, and could possibly replace bone marrow biopsy. Both intravenous regimens are given every two weeks for six months. There are currently 51 patients being followed on this protocol, with two to three patients in active treatment on any given month. This treatment regimen is also highly effective, so patients are seen for years in long-term follow-up.
4. Protocol 10-C-0066: "Collection of human samples to study hairy cell and other leukemias, and to develop recombinant immunotoxins for cancer treatment." The primary objective of this study is to allow the collection of a variety of clinical samples, including blood, urine, lymphopheresis samples, and other tissues, to better understand the disease processes that are being studied, or to determine eligibility and/or optimal timing for clinical testing. All patients who are under the care of Dr. Kreitman are initially signed up to this protocol for testing. The samples are used to study antibodies made against immunotoxins, quantifying tumor antigens by flow cytometry and other methods. They are also used to test how well recombinant immunotoxins and other agents kill tumor cells ex vivo, and molecularly characterizes malignant B-cells by sequencing their immunoglobulin rearrangements and other genes.
5. Protocol 13-C-0106: "A Pivotal Multicenter Trial of Moxetumomab pasudotox in Relapsed/Refractory Hairy Cell Leukemia." Moxetumomab pasudotox is a recombinant immunotoxin containing an Fv fragment of an anti-CD22 monoclonal antibody and truncated Pseudomonas exotoxin that has demonstrated a high complete remission rate in patients with chemo-resistant hairy cell leukemia. This treatment has also shown activity in pediatric acute lymphoblastic leukemia. It is an intravenous drug, given on an inpatient basis on days one, three, and five every 28 days. There are currently 26 patients being followed on this protocol, including three patients in active treatment at the time of this report. Patients treated on this protocol are anticipated to maintain a complete response without minimal residual disease and be seen in follow-up for years to come.
6. Protocol 14-C-0004: "A Multicenter Phase II Study of the Bruton's Tyrosine Kinase Inhibitor PCI-32764 for Treatment of Relapsed Hairy Cell Leukemia." This is a targeted oral therapy for patients relapsing after treatment with purine analog, or for patients who are unable to receive a purine nucleoside analog. There are currently 10 patients on this protocol in active treatment. Ibrutinib is taken on a continuous basis until unacceptable toxicities or disease progression. The first patient was enrolled in March 2014, and remains on treatment with ongoing partial response and essentially no toxicities noted to date.
7. Protocol 14-C-0131: "A Phase II, open label study in subjects with BRAF V600E-mutated rare cancers with several histologies to investigate the clinical efficacy and safety of the combination therapy of dabrafenib and trametinib." The combination of both dabrafenib plus trametinib has indicated increased efficacy in tumors positive for BRAF V600E mutations over both monotherapies. The primary objective of this protocol is to determine the overall response rate as measured by established response criteria for hairy cell leukemia. This treatment has been shown to be highly effective in treating multiply relapsed or refractory hairy cell leukemia, albeit with some toxicity challenges. LMB currently has 11 patients in active treatment, including one patient with anaplastic thyroid cancer who was transferred to this study when their doctor left the NIH last year.
8. Protocol 06-C-0150: "A Phase II clinical trial of anti-tac(Fv)-PE38 (LMB-2) immunotoxin for CD25 positive hairy cell leukemia." The primary objective of this trial is to determine the response rate of LMB-2 in patients with CD25-positive hairy cell leukemia. The secondary objectives include determining response duration and describing how the development of neutralizing antibodies affects blood levels of LMB-2 and toxicity. LMB currently has 12 patients in follow-up, with two patients identified for treatment in the next two months.

Patients are still seen semi-annually or annually for routine follow-up on an inactive protocol (07-C-0130, "A Phase I multicenter, dose escalation study of CAT-8015 in patients with relapsed or refractory hairy cell leukemia"). Many of the patients treated on this protocol have had an excellent response to treatment and remain in complete or partial response. There are 27 patients who are monitored closely for evidence of relapsed disease, and are then evaluated for potential participation in another active protocol.

New and follow-up patients often arrive with a variety of clinical challenges and co-morbidities that impact their ability to participate in a clinical trial. The Senior Nurse Practitioner continues to decrease the impact of these challenges by thoroughly reviewing patients' clinical records, interval histories, participating in phone screen consult prior to clinic visits, and reviewing all patients' medication lists for possible interactions or contraindications. This has resulted in a smoother clinic experience for the medical providers (Fellows, M.D.s), nurses, and, most importantly, the patients who come to the facility for evaluation and treatment.

As the Phase III immunotoxin moxetumomab protocol winds down and completes accrual, LMB is working towards development of an expanded access protocol for this highly successful treatment, as well as a new protocol with moxetumomab and rituximab for newly diagnosed hairy cell leukemia, and hairy cell leukemia variant patients. LMB is also discussing development of a PCR treatment regimen with pentostatin, rituximab, and cyclophosphamide with moxetumomab.

Host Virus Interaction Branch, HIV Drug Resistance Program

As the clinical arm of the HIV Drug Resistance Program, the Host-Virus Interaction Branch (HVIB) conducts fundamental studies on the nature of HIV drug resistance in vivo. Ongoing studies are focused on characterizing the replicating population size and genetics of HIV in infected individuals before, during, and after antiretroviral therapy (ART); defining the genetic mechanisms, kinetics of emergence and decay, and clinical consequences of HIV drug resistance; identifying the tissue and cellular sources of persistent viremia despite suppressive ART; and testing novel therapeutics to reduce persistent viremia and deplete HIV reservoirs.

The Clinical Retrovirology Section of the HVIB directs protocol development, regulatory affairs, patient recruitment, and sample collection efforts for the HVIB at the NIH Clinical Center, in collaboration with the AIDS clinical research programs of the NCI HIV and AIDS Malignancy Branch, NIAID, and the Critical Care Medicine Department. The Clinical Retrovirology Section also conducts fundamental studies of HIV pathogenesis in vivo, including studies of HIV genetic variation and the emergence of antiretroviral drug resistance. CMRP provides a protocol nurse coordinator to support these efforts.

The protocol nurse coordinator completed study enrollment on two protocols: 11-I-0057, Effect of Interferon Alpha 2b Intensification on HIV-1 Residual Viremia in Individuals Suppressed on Antiretroviral Therapy; and GUCHEK 13-I-0062, A Double Blind Randomized Placebo Controlled Study Examining the Effects of a Non-Absorbable (Rifaximin) Antibiotic on the Chronic Immune Activation Observed in HIV-Infected Subjects. The last patients enrolled in 13-I-0062 will complete all study requirements by the end of July 2016. The patients in 11-I-0057 will complete all study related requirements by the end of November 2016.

The protocol nurse coordinator manages all aspects of three active protocols in the Clinical Retrovirology Section. These activities include coordinating patient visits to NIH, directing patients to admissions, consenting the patients for screening, preparing monthly patient calendars, and acting as a liaison between patients and health care providers.

The protocol nurse coordinator continued work on the protocol titled Localizing Cellular Sources of HIV Infected Cells Persisting in Lymphoid Tissue during Combination Antiretroviral Therapy. This study should be open by the end of FY2016.

Molecular Imaging Program

The goal of the Molecular Imaging Program (MIP) is to develop targeted imaging methods that accelerate the development of cancer therapies. This program performs translational research in targeted cancer imaging for the purposes of early tumor detection and characterization, treatment monitoring, and drug development. MIP investigates cancer therapies in both the pre-clinical and clinical realms, providing benchtop-to-bedside research. CMRP provides a dedicated team of professionals to support MIP.

A positron emission tomography (PET) physicist performs radiation dosimetry for clinical trials, credentials the PET/CT scanner for clinical trial experiments, performs quantitative analysis, and solves technical image-quality problems.

A senior chemist provides technical knowledge regarding the clinical development of a broad array of potential imaging agents, including new chemical entities, and nano- and biotechnologies. The senior chemist investigates the feasibility of labeling new imaging agents based on their chemical properties and determines translational feasibilities, including the viability of commercial production from a chemistry development viewpoint. The senior chemist also defines required experiments for preclinical testing, and provides process development and analysis of the data, to include in presentations and publications.

PET/CT technologists perform highly skilled PET/CT scans on patients involved in clinical trials. The technologists are instrumental in writing policy relevant to PET/CT and maintaining quality assurance (QA) for radiation safety. CMRP staff has met the rigorous credentialing requirements

necessary to function as authorized users of radiopharmaceuticals. This allows direct support to the PI and the entire department. The PET/CT technologists scan an average of 30–40 patients a week.

The MRI/CT/radiology technologist is credentialed in three modalities and is a candidate for PET certification training. This technologist is responsible for developing and implementing SOPs related to MRI contrast and delivery.

The clinical health associate assists with all patient care duties, accruing and monitoring patients during protocol participation, providing patients with appointment reminders, and documenting all patient information (contact, follow-up and off-study calls, off-study notes, PET research notes, vital signs, notes pertaining to incidents within the clinic) in the electronic medical record and the imaging trial database.

The PCC schedules an average of 30–40 patients per week. Other responsibilities include serving as an interpreter for Spanish-speaking patients and interfacing with other branches, such as urology, to coordinate referrals to the department. In addition, the PCC provides administrative support to the PI and clinical trials clinic.

CMRP staff perform all other support tasks related to the day-to-day activities of the MIP clinical trials. For example, CMRP staff coordinated efforts with the NCI Office and Space Facilities Management to prepare construction for installing the new PET/CT scanner that MIP purchased.

Work on analyzing the imaging data for clinical protocol 14-C-0140, A Pilot Study of F18-DCFBC PET/CT in Prostate Cancer, is ongoing. The protocol has three arms: localized prostate cancer, biochemical recurrence, and metastatic disease. The images and data acquired for each are undergoing analysis, and separate manuscripts are being developed.

A manuscript on the results of clinical trial 11-C-0061, A Phase I Study of Adults with Hormone Receptor-Positive Tumors, is being finalized for journal submission.

The multi-year comprehensive Cooperative Research and Development Agreement (CRADA) between NCI and MedImmune, Inc. to investigate defined research avenues of common interest has completed its first year. As part of this CRADA, MIP completed preclinical in vitro and in vivo imaging investigations on two MedImmune antibodies that are being developed for two distinct therapeutic targets. During FY2016, MIP successfully met all of its goals under the CRADA in the allotted time frame. Planning for year two is now under way.

A collaborative effort between MIP and a commercial pharmaceutical partner has progressed through preclinical animal testing. Under this agreement, MIP will transfer and develop the 89Zr-anti-PD-L1 anti-checkpoint antibody for clinical use.

MIP has also established a collaborative agreement with the Laboratory Animal Sciences Program (LASP) at Leidos Biomed to evaluate agents in all appropriate preclinical animal models, including histological testing.

Genitourinary Malignancies Branch

The Genitourinary Malignancies Branch (GMB) focuses on investigating the biology of genitourinary cancers, developing new strategies for treating those cancers, and evaluating new therapeutic approaches through science-driven clinical research. Clinical trials investigate novel approaches in immunotherapy, hormonal therapy, chemotherapy combinations, and small-molecule targeted therapy.

Bladder Cancer Medical Oncology currently has a Phase II protocol (12-C-0205) with Cabozantinib that completed accrual in January 2016; GMB also has a Phase I protocol (15-C-0160) with Cabozantinib with Nivolumab and Ipilimumab that has enrolled 30 patients; this is a multi-facility protocol with other sites at the Ohio State University, Rutgers University, and City of Hope.

A credentialed CMRP physician assistant (PA) provides direct patient care support to the GMB inpatient and outpatient clinics by performing routine and history exams, interpreting lab results, and managing patient treatment plans for patients. The PA also conducts follow-up phone calls and daily walk-in clinic visits for patients on standard of care treatments and patients on protocols. During FY2016, the PA was asked to help with creating the Multidisciplinary Bladder Cancer website, which will be updated to show new clinical trials available with Medical Oncology, Urology Surgery, and Radiation Therapy branches.

In September 2015, the PA was asked to manage weekly multidisciplinary bladder conferences with the urology surgery, radiation oncology, medical oncology, pathology, and radiology teams. Patient synopses are compiled for discussion about treatment plan recommendations.

The PA also reviews new patient data, coordinates with the medical oncology referral team for screening or second opinion evaluations, and follows up with the referral team and research nurse to confirm receipt of pathology and radiology reports prior to a patient's first visit.

Direct patient contact has improved the overall clinical approach and the PA's creation of new patient/referral synopsis assists the attending physician and medical oncology support staff address the needs of patients.

Laboratory of Tumor Immunology and Biology

The Laboratory of Tumor Immunology and Biology (LTIB) functions as a multidisciplinary and interdisciplinary translational research effort with the goal of developing novel immunotherapies for human carcinomas, not only as monotherapies, but in combination with other immune-mediating modalities and conventional or experimental therapies as part of an immuno-oncology programmatic effort. Within this effort are several research groups: a clinical trials group, two independent investigators, collaborations with intramural and extramural scientific and clinical investigators, and collaborations with investigators in the private sector. The

program takes advantage of the uniqueness of the NCI intramural program in that it spans high-risk basic discovery research in immunology and tumor biology through preclinical translational research to paradigm-shifting clinical trials. Focus is placed on the design and development of novel recombinant vaccines and immunomodulators that can be used in clinical studies at numerous institutions and do not involve costly and labor-intensive ex vivo manipulations that can be carried out in only one or two centers. This work is accomplished though CRADAs with private-sector partners who provide agents for preclinical studies in appropriate animal models.

CMRP supports LTIB by providing a PA and a research nurse to work within the Clinical Trials Group. These professionals actively support ongoing LTIB and GMB protocols. The PA and the research nurse successfully collaborate with LTIB members as well as GMB members, as much of LTIB and GMB work is intertwined. They attend inpatient rounds, participate in study-related conferences and meetings, assure continuity of patient care through routine communications with the inpatient team, and support adherence to protocols.

The PA works together with the clinical team to provide clinical care for study participants. She performs medical evaluations, monitors medical status side effects related to the study interventions, and provides care for acute and non-acute clinical problems. She is also actively involved in the screening and recruitment process for all LTIB clinical trials.

The research nurse independently coordinates patient visits to NIH, consents patients for screening, acts as a liaison between the patient and the providers, coordinates specimen pickups, and records all unanticipated problems and adverse events (AEs).

Medical Oncology Service

The Medical Oncology Service's major functions are to translate laboratory observations into the clinical setting and to conduct clinical research and training. The program's goals are to develop novel therapeutic research strategies for cancer treatment and to test those strategies by conducting clinical research in medical oncology across a spectrum of diseases and disease mechanisms; to provide clinical care to adult cancer patients enrolled in research protocols, including inpatient and outpatient care services; to support the clinical research effort emanating from principal investigators in CCR laboratories and branches; and to train physician-scientists in a laboratory-to-clinic translational research setting to promote the development of their expertise in medical oncology research and to support their board certification by the American Board of Internal Medicine.

Currently, CMRP has four nurse practitioners and one physician extender supporting the Medical Oncology Service. These practitioners staff the inpatient service at the NIH Clinical Center and provide direct care to patients hospitalized for the management of acute health conditions, as well as support and oversight of protocol-related treatments, tests, and procedures. These individuals provide high-level comprehensive inpatient care to support, streamline, and enhance the work of the Medical Oncology Service.

The CMRP inpatient medical oncology team continues to support and provide in-hospital care for patients being treated through a growing number of protocols. This year, the following protocols were added:

- 15-C-0150: A Phase I/II Trial of Topotecan with VX970, an ATR Kinase Inhibitor in Small Cell Lung Cancer
- 15-C-0160: A Phase I Study of Cabozantinib Plus Nivolumab (CaboNivo) Alone or in Combination with Ipilimumab (CaboNivoIpi) in Patients with Advanced/Metastatic Urothelial Carcinoma and Other Genitourinary Tumors
- 15-C-0178: A Phase Ib, Dose Escalation, Multiple Dose Trial with HuMax-IL8 in Patients with Metastatic or Unresectable, Locally Advanced Malignant Solid Tumors
- 16-C-0013: An Open-Label, Phase I Study of the Safety and Immunogenicity of JNJ-64041757, a Live Attenuated Listeria Monocytogenes Immunotherapy, in Subjects with Non-Small Cell Lung Cancer Top of Form 1
- 16-C-0107: A Phase I/II Trial of CRLX101, a Nanoparticle Camptothecin with Olaparib, in Patients with Relapsed/Refractory Small Cell Lung Cancer

Neuro-Oncology Branch

Malignant brain tumors are the most common cause of cancer-related deaths in adolescents and young adults aged 15–39. The Neuro-Oncology Branch (NOB) Brain Tumor Clinic is a joint program of NCI and the NINDS. NOB comprises a multidisciplinary team of physicians, healthcare providers, and scientists who are dedicated to developing new therapies and improving outcomes for patients with primary brain and spinal cord tumors. It provides cutting-edge neurosurgery and radiation therapy based on the genetic characteristics of specific tumors. Through the clinic, located at the NIH Clinical Center, patients are given expert evaluations, examinations, tests, and state-of-the-art imaging (MRI and PET scans) during and after treatment, and are given access to new therapies and procedures not available elsewhere.

During this reporting period, CMRP recruited and hired one PCC to support the expanding clinic efforts within NOB. The PCC is currently a member of a CCR Referrals work group, assembled in January 2016. This group is planning and providing input to software and database developers working on a new computer system that will be used to organize, track, and extract patient data and research information for the NOB. The PCC is also currently a member of an interdisciplinary task force assembled by the NOB chief in April 2016 to facilitate and streamline the new patient intake process.

Brain Tumor Trial Collaborative

NOB is developing a world-class clinical and translational research program—the Brain Tumor Trial Collaborative (BTTC), funded in part by a philanthropic partner, Head for the Cure (HFTC) Foundation. BTTC was created as a network of 22 medical institutions with the expertise and desire to participate in state-of-the-art clinical trials investigating new treatments for malignant brain tumors. In 2015, NCI transitioned the BTTC Coordinating Center from MD Anderson to the NCI CCR, which serves as the lead institution and provides the administrative infrastructure, clinical database, and oversight for the collaborative. Leidos Biomed began to provide dedicated staff to support and oversee the collaborative in June 2015.

CMRP supports the clinical and administrative infrastructure necessary to transition all operations from MD Anderson to NCI. A clinical project manager, a protocol coordinator, and a clinical research nurse support the comprehensive activities.

In FY2016, CMRP staff supported the pre-negotiation activities and subsequent management of several research agreements to support BTTC: 1) agreement with UVI to provide drug distribution to the network sites, 2) consulting agreement with a biostatistician with statistical expertise to design innovative Bayesian adaptive clinical trials to support the BTTC clinical trials, and 3) consulting agreement with an experienced communications specialist to provide community outreach, website development and maintenance, communications, and publications management for BTTC and its network of medical institutions. Additional agreements will be negotiated with each of the BTTC network accrual sites to allow for reimbursement of patient accrual and related costs to begin on one or more clinical trials; the timeline is dependent on new CRADA agreements being established between NCI and protocol-specific pharma partners. The BTTC team is working with the CCR Technology Transfer Office to execute these CRADA agreements and to establish reliance agreements with BTTC member institutions. Both agreements must be in place before the Leidos Biomed agreement can be finalized; expected by September 2016.

Staff also supported the NCI CCR with protocol development and navigation efforts in order to obtain IRB approval for the BTTC clinical trials, and with testing/building the BTTC studies into the NIH clinical and laboratory databases, C3D and Labmatrix.

During the reporting period, the clinical project manager partnered with members of the NOB team to successfully plan, conduct, and execute the annual BTTC meeting held at NIH in October 2015. The annual meeting served as a BTTC relaunching event with CCR, as NCI assumed the role as lead institution of the collaboration. Attendees of the meeting were principal investigators of all BTTC member institutions, members of the CMRP and NOB teams, and HFTC Board of Directors.

The BTTC project team launched the BTTC web-based communication on the CCR wiki page and continues to collaborate with the HFTC team to update page content. The team also updated the BTTC Operations Manual to reflect the operations, rules, and regulations of NCI; this manual was distributed to BTTC member institutions. Both the BTTC Operations Manual and Consortium Agreements were distributed to sites in November 2015. Significant progress has been made to transition the protocols from MD Anderson to NCI so that sites can obtain their IRB approvals, while the BTTC network sites have begun to submit new concepts for approval.

Vaccine Branch

Cancer and HIV are both chronic diseases that suppress and evade the immune system. By combining expertise in both cancer and retroviral vaccines, the Vaccine Branch (VB) aims to promote the cross-fertilization of ideas and progress in both areas in a unique way that is not duplicated elsewhere. The branch conducts a program of clinical and laboratory research designed to: (1) elucidate basic mechanisms of immune response and molecular virology, and (2) apply these to the design and development of vaccines and immunotherapy for the prevention and treatment of cancer and AIDS, as well as viruses that cause cancer.

The VB carries out studies on: (1) the mechanisms of T-lymphocyte activation and regulation; (2) cancer immune surveillance, (3) mucosal immunity, (4) retroviral molecular biology and pathogenesis (including transcriptional and post-transcriptional regulation of retroviruses involved in causing cancer or AIDS), (5) regulating cellular gene expression, (6) immune responses to retroviruses, and (7) strategies for rational vaccine design. The branch utilizes these findings to design novel vaccines for cancer, HIV, and cancer- and AIDS-associated viruses.

The PCC provides daily clinical support in the following areas: patient communications, pre-screening information communication, and pre-screen packets. The PCC assists in scheduling laboratory and diagnostic exams, registering patients for clinical protocols, and submitting patient specimens and other medical documentation to appropriate ancillary departments.

Currently, the VB has three active protocols: 13-C-0016, 15-C-0075, and 15-C-0076, with a total enrollment of 36 subjects. The protocol requirements are complex to manage because they are custom-made dendritic cell vaccines produced by the Department of Transfusion Medicine. Manufacturing is a five-day process with a stringent set of criteria to produce viable cells.

Dermatology Branch

The Dermatology Branch (DB) conducts clinical and basic research to study the etiology, diagnosis, and treatment of inflammatory and malignant diseases involving the skin, and the host's response to these diseases. Research involves biochemical as well as

biological studies of skin, and is carried out in laboratories and in the clinic. Research areas of interest include characterizing skin as an immunological organ and defining the role of dendritic cells and molecules expressed by these cells in the generation of skin-centered immune responses. There is significant emphasis on inflammatory skin diseases in mice and humans, on the cutaneous microbiome in normal individuals, and on the cutaneous microbiome in patients with atopic dermatitis and selected primary immunodeficiencies. Other investigations involve long-term clinical and laboratory studies of DNA repair, skin cancer risk, and developmental abnormalities in cohorts of patients with xeroderma pigmentosum or trichothiodystrophy.

DB's laboratory research includes studies of skin stem cells and cutaneous malignancies, including Merkel cell carcinoma. DB's consultation service is one of the busiest clinical services in CCR and is responsible for all outpatient and inpatient dermatologic patient care delivered at NIH.

CMRP provides clinical and medical support services to DB that include patient recruitment, enrollment, and screening, and various services related to research and regulatory activities for interventional and biospecimen acquisition protocols. CMRP support involves monitoring and maintaining clinical research protocol compliance, assisting in data collection and analysis, conducting updates on patient care with clinical staff and community physicians, and consulting with other healthcare providers to meet various patient needs.

The CMRP clinical research nurse currently supporting DB is primarily working on one protocol at the main NIH/NCI campus. Patients are recruited from various oncology teams throughout NCI (there are nine separate oncology patient populations at this time), focusing on patients starting immunotherapy trials. A small subset of Neuro-Oncology patients on a natural history study is also being enrolled.

The CMRP clinical research nurse also assists with coverage for additional protocols (12-C-0159, 96-C-0102, and 08-HG-0059) while the team seeks a replacement for a departing NCI clinical research nurse.

Since the clinical research nurse began supporting DB, accrual of oncology patients has increased almost 50% and accrual is anticipated to continue to increase in the coming months as we begin to collaborate with more oncology teams and patient populations.

Women's Malignancies Branch

The Women's Malignancies Branch (WMB) conducts basic, translational, and clinical research on the mechanisms of cancer with emphasis on those that only or primarily affect females such as breast and ovarian cancer. Research in the laboratories of the Branch covers subjects including growth factor receptor signaling, cell survival and the induction of apoptosis, metastasis, and DNA repair and cell cycle check points. The research links basic and translational experimental cancer biology and clinical investigation. In June 2016, CMRP began to support the branch by providing a clinical research nurse to assist in activities.

Support Provided by the Applied and Developmental Research Directorate

The Applied and Developmental Research Directorate (ADRD) has dedicated laboratories and support functions funded by the Center for Cancer Research (CCR) to provide clinical trial support, including specimen processing and testing. Multiple laboratories provide testing support that is used in making patient treatment decisions and is regulated under the Clinical Laboratory Improvement Amendments (CLIA). Both the Clinical Support Laboratory and Laboratory of Cell-Mediated Immunity offer testing services through the Shared Services system, and those activities for non-CCR investigators are described in relevant sections of this report.

Clinical Support Laboratory

The Clinical Support Laboratory (CSL) is a CCR-funded core that was established to provide multifaceted clinical trial support to CCR investigators. CSL is organized into three laboratory sections. The Clinical Monitoring section is responsible for receipt, processing, cryopreservation, and database entry of clinical samples. The Lymphokine (Biomarker) Testing section performs enzyme-linked immunosorbent assay (ELISA), electrochemiluminescent multiplex assays, and bioassays for a wide range of biomarkers. The Flow Cytometry section performs immunophenotyping, intracellular staining, tetramer staining, and other flow cytometry–based assessments of cell activity, as requested. While the bulk of CSL's activities are in support of clinical trials, the laboratory also performs research sample testing in support of CCR principal investigators, with support requests submitted primarily through the Core Service Accessioning System (CSAS) request system. The laboratory works with investigators to ensure that critical clinical samples received late on Fridays or on weekends are processed and/or tested in a timely manner. The laboratory is CLIA certified for performing several high-complexity assays.

- Pediatric Oncology Branch Support
  - The laboratory received over 2,500 samples of whole blood, serum, plasma, leukapheresis products, elutriation cell fractions, bone marrow, cerebrospinal fluid, and DNA PAXGene from 13 clinical trials, with approximately 9,500 vials stored.
  - On nine occasions, the laboratory was asked to perform immediate cytokine testing in support of a Pediatric Oncology Branch clinical trial. Each sample submitted was tested in a panel of five single-cytokine ELISAs. A total of

12 samples were submitted, and test results were provided to the requester on the same day that the testing was performed.

- In response to 11 requests for testing, CSL performed multiplex testing of human and mouse serum/plasma or tissue culture samples. A total of 933 samples were submitted for testing, with 9,066 data points evaluated.
- At the request of Dr. Lee Helman, the laboratory worked with the Biorepository to perform quality control (QC) and discard of the I99 Source Code collection that contained over 140,000 vials.

- Surgery Branch Support. The laboratory processed 800 blood samples from branch-sponsored trials received in Cell Preparation Tube™ vacutainer tubes for isolation and cryopreservation of mononuclear cells, with 3,020 vials produced. The laboratory responded to multiple investigator requests to pull samples from the repository for return to the Surgery Branch for testing.
- Lymphoid Malignancies Branch Support
  - In support of the Cytokine Immunology and Immunotherapy Section, the laboratory received 273 samples in support of nine clinical trials, including three offsite trials, with 1,074 vials of serum or cells cryopreserved.
  - In support of the Lymphoma Therapeutics Section, the laboratory processed 553 samples in support of 10 clinical trials, with approximately 2,300 vials of serum stored.
  - The laboratory provides testing for anti-daclizumab, anti-human Mikβ1, detection of human Mikβ1, and anti-interleukin (IL)-15 as CLIA-regulated assays to support branch trials. Some testing is also identified as "research only." Each of these assays requires testing each sample at multiple dilutions. Sample testing included the following:

Assay	# Test Requests	Total Samples
Anti-daclizumab	1	1
Anti-huMikβ1	14	15
HuMikβ1	1	2
Anti-IL-15	13	72

  - Additional biomarker support included the following:

Assay	# Test Requests	# Samples	Data Points
IL-15 PK	9	573	1,146
IL-18	5	153	306
IL-6 Receptor	5	153	306
sIL2Ra	6	119	238
Multiplex	12	546	3,276

- Vaccine Branch Support. The laboratory received 92 samples from two clinical trials, with 1,193 vials stored.
  - The laboratory worked with Dr. Jay Berzofsky and Fisher BioServices to complete the transfer of over 25,000 vials of clinical material to Dr. Samir Khleif, director, Georgia Reagents University Cancer Center, under a Material Transfer Agreement.
  - In support of Yellow Task (YT) 13-074 and Dr. George Pavlakis, the laboratory performed an IL-15 bioassay to assess the biological activity of an assay standard and a toxicology lot of heterodimer, as well as the cGMP IL-15 heterodimer drug product to be used for clinical trials. Assays were also performed to evaluate product stability and to confirm assay performance. A total of 19 assays were set up, testing 19 samples on 71 test plates. Maintaining this assay required long-term culture of multiple passages of the NK92 cell line.
  - Further testing in support of hetIL-15 product development included 20 test plates to evaluate a neutralization assay for the detection of antibodies that inhibit the bioactivity of hetIL-15, as well as 2 ELISA plates for preliminary screening of patient samples for anti-Het-IL15.
- Laboratory of Tumor Immunology and Biology Support
  - The laboratory provided support to 21 branch trials, including several trials conducted at off-site locations, which required additional coordination of couriers and close interaction with clinical staff. One trial required specialized cryopreservation media, while another required segregated sample processing to address Institutional Biosafety Committee (IBC) safety concerns related to the receipt of swabs obtained from vaccinia vaccination sites. A total of 3,350 samples were received or isolated, including peripheral blood mononuclear cells (PBMCs) from blood and leukapheresis products, serum, plasma, urine, and swabs, with over 35,000 vials of clinical materials stored into eight different repository sample collections.

  - In support of CSAS-16483, the laboratory performed multiplex or singleplex electro-chemiluminescence testing on 152 samples, resulting in the evaluation of 2,584 data points.
  - Multiplex testing of 38 mouse samples resulted in the evaluation of 380 data points (CSAS-17453).
- Molecular Imaging Program Support. In response to CSAS-16794, the laboratory performed 4 assays to evaluate endotoxin levels in radiolabeled test products, with a total of 11 samples tested.
- Laboratory of Molecular Immunology Support. In response to multiple CSAS requests for ELISA and multiplex testing of human and mouse samples, a total of 4 sets of assays were performed on 321 total samples, resulting in the evaluation of 1,405 data points.
- Urologic Oncology Branch Support. In response to CSAS-17085, the laboratory expanded six cell lines for cryopreservation in order to distribute to branch collaborators. In response to CSAS-17446, the laboratory established 10 Epstein-Barr virus–transformed cells lines from clinical samples.
- Thoracic and Gastrointestinal Oncology Branch Support. In response to CSAS-17299 and -17764, the laboratory performed testing to optimize an assay for the detection of antibodies to mesothelin, and to establish a normal donor range for the assay and use the assay to evaluate patient samples from two studies.
- Laboratory of Human Carcinogenesis. In response to CSAS-16116 from Dr. Brid Ryan, the laboratory performed evaluation of a custom 33-plex cytokine panel on 367 samples for 12,111 test results.

Blood Processing Core

Leidos Biomedical Research provides core staffing support to the Blood Processing Core (BPC), with additional staffing provided from the NCI Clinical Pharmacology Program. Core personnel also provided weekend, holiday, and after-hours on-call support for human sample processing.

Laboratory of Cell-Mediated Immunity

The Laboratory of Cell-Mediated Immunity (LCMI) is a CCR-dedicated laboratory that offers testing support to CCR investigators and the research community at large through the Shared Services system. Dr. Anatoli Malyguine, head of LCMI, retired at the end of fiscal year (FY) 2014, and Dr. Ludmila Krymskaya was hired in FY2015. The laboratory relocated from Building 560 to Building 469, allowing for more coordination between LCMI and CSL activities. It is anticipated that the two labs will merge at the beginning of FY2016.

- Support to the Laboratory of Immunoregulation. LCMI staff analyzed 18 ELISPOT plates at the request of Dr. Arthur Hurwitz (YT04-154) and Dr. Katie Stagliano (CSAS-17350).
- Support to the Vaccine Branch
  - LCMI staff analyzed 10 ELISPOT plates at the request of Dr. George Pavlakis (CSAS-16851, -16928, -17028).
  - LCMI staff performed 600 ELISPOT tests at the request of Dr. Lauren Wood (CSAS-17320 and -17692).
  - LCMI staff performed 210 in vitro stimulations at the request of Dr. Masaki Terabe (YT14-130). As part of the same request, CSL evaluated in vitro–stimulated cells and tetramer expression.

Laboratory of Molecular Biology

The Human Cancer Immunotoxin Therapy Assay Support and Development Laboratory provides dedicated laboratory support to Dr. Ira Pastan, co-chief of the Laboratory of Molecular Biology (LMB/NCI), Dr. Robert Kreitman, head of the Clinical Immunotherapy Section (LMB/NCI), and Dr. Raffit Hassan, co-chief of the Thoracic and Gastrointestinal Oncology Branch and head of the Solid Tumors Immunotherapy Section (NCI). This includes both support for investigator-initiated studies (non-CLIA) and NCI-sponsored clinical trials (CLIA). The laboratory is CLIA certified as mandated by the Cancer Therapy Evaluation Program (CTEP) and actively supports CTEP-approved Investigational New Drug (IND) immunotoxin clinical trial protocols. The support provided for LMB investigators includes immunotoxin cell-based neutralization assays, and cell-based assays to measure immunotoxin concentrations present in patient serum and plasma samples drawn during immunotoxin treatment. Patients who have exhausted the benefit derived from all other standard approved cancer therapies are identified by NCI investigators for potential immunotoxin therapy. The laboratory performs immunotoxin-specific, cell-based neutralization assays to screen these patients in order to detect the presence of a pre-existing antibody for the particular immunotoxin, which would exclude them from treatment. Once patients have been treated with a particular immunotoxin, they must be reevaluated at defined intervals to determine the degree of their immune response to the immunotoxin, and this testing determines their continued treatment cycle eligibility. The SS1 immunotoxin treatment schedule is time sensitive, and the SS1 neutralization assays must be performed and their results sent for analysis in order to avoid violating the approved protocol treatment schedules. Fewer samples are being run per assay because of the time sensitivity associated with these assays, which prevent sample batching. When a patient is treated with an immunotoxin infusion, during the course of treatment, blood samples are obtained at specified time intervals for later testing in an immunotoxin-specific, cell-based

pharmacokinetic (PK) assay, to determine the quantity and half-life of the drug present in the patient's circulation.

Immunotoxins being evaluated clinically include: LMB2 for certain lymphomas; HA22, which is a high-affinity immunotoxin for the treatment of certain lymphomas and hairy cell leukemia in adult and pediatric patients; and SS1 immunotoxin, which is specifically designed for the treatment of mesothelioma, a solid tumor of the lung. This year, the Phase IV clinical trial of the HA22 immunotoxin continued in conjunction with MedImmune and NCI. Also this year, a new, less immunogenic, experimental SS1 immunotoxin was tested after development of a new in vitro cell assay. This assay is more complex than the one currently in use and uses a different cell line that requires a 42-hour intracellular incubation for the immunotoxin to be processed and for the cell to be killed. Several assays were completed, and the resulting data was evaluated and submitted to NCI investigators.

SIGNIFICANT ACHIEVEMENTS

- LMB2 assay support. Twenty-six immunotoxin neutralization assays were performed with 86 patient samples, and two PK assays were performed on 120 patient samples. These assays are cell-based, multiple-microplate radiological assays. A total of 376 patient serum and plasma samples were processed and frozen for future study.
- HA22 assay support. Thirty-six immunotoxin neutralization assays were performed on 138 patient samples. These assays are cell-based, multiple-microplate radiological assays. A total of 414 patient samples were tested in 10 PK assays, and 420 patient serum samples were processed and frozen for future studies.
- SS1 assay support. Forty-six immunotoxin neutralization assays were performed with 68 patient samples, and 20 PK assays were performed on 240 patient samples. These assays are cell-based, multiple-microplate radiological assays. A total of 816 SS1 study serum samples were processed and frozen for future study.
- U.S. Food and Drug Administration (FDA) validation of LMB2 immunotoxin (stability and potency). The laboratory continued long-term stability testing of LMB2 immunotoxin lots that are used in patient treatment. LMB2 immunotoxin potency stability assays were performed using a specific cell-based microplate assay format, and the results were submitted to the FDA in support of the LMB2 IND protocols. One LMB2 assay was performed on two samples. These are all cell-based, multiple-plate radiological assays.
- FDA validation of SS1 immunotoxin (stability and potency). The laboratory performed long-term stability testing on one SS1 immunotoxin lot that is used in patient treatment. SS1 immunotoxin potency stability assays were performed using a specific cell-based microplate radiological assay format, and the results were submitted to the FDA in support of the SS1 IND protocol.
- CLIA recertification. The laboratory was inspected on January 21, 2015, and has received CLIA recertification for two years.

HIV and AIDS Malignancy Branch

The AIDS Monitoring Laboratory (AML) provides dedicated clinical trials support to the HIV and AIDS Malignancy Branch:

- In support to Dr. Robert Yarchoan**,** AML monitored nine active clinical research protocols for patients with HIV/AIDS, AIDS-related malignancies, and viral-induced tumors. The laboratory is responsible for the receipt, processing, and cryopreservation of clinical specimens; for performing immunophenotypic analysis of whole blood specimens; and for performing ELISA assays for a wide range of serum/plasma biomarkers. This work resulted in the processing of 136 whole blood specimens, 250 serum specimens, 480 plasma specimens, 4 pleural effusions, and 76 urine specimens. AML performed 31 CLIA-regulated cell immunophenotype determinations by flow cytometry and 570 cytokine measurements. AML cryopreserved and stored 364 vials of patient PBMCs, 2,428 vials of serum, 52 vials of pleural effusion fluid, 696 vials of urine, 2,962 vials of plasma, and 926 vials of PBMC cell pellets. AML coordinated six shipments of clinical specimens to investigators and institutions located at various domestic and international sites.
- In support to Dr. Thomas Uldrick, NCI, the AML Clinical Group developed a custom nine-color immunophenotyping panel to perform a detailed analysis of monocyte subpopulations in blood of patients enrolled in a Phase-I trial of pomalidomide in combination with liposomal doxorubicin in patients with advanced or refractory Kaposi sarcoma (KS). Monocytes have been shown to be relevant in immune activation and in the setting of HIV infection, monocyte phenotypes correlate well with soluble biomarkers of clinical relevance, such as interleukin (IL)-6, CRP, and D-dimer. Monocytes have strong relevance in atherosclerosis and monocyte changes are attractive targets for intervention and for study endpoints. During FY2016, monocyte subpopulations were analyzed in 15 patient specimens.
- In support to Dr. Thomas Uldrick, NCI, the AML Clinical Group examined programmed death-ligand 1 (PDL-1) expression on monocytes and neutrophils in whole blood using a custom three-color immunophenotyping panel. PD-L1, also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1), is a protein that, in humans, is

encoded by the CD274 gene. PDL-1 has been thought to play a major role in suppressing the immune system during particular events such as autoimmunity. In mouse models, activated monocytes have been shown to greatly up-regulate PD-L1. PD-L1 has been shown to act as a positive co-stimulatory molecule in intracellular infection. During FY2016, AML analyzed PD-L1 expression in 15 patient specimens.

- In support of Dr. Thomas Uldrick, NCI, the laboratory measured a panel of 30 cytokine biomarkers in 19 patients with anal cancer.
- In support of Dr. Hiroaki Mitsuya**,** AML performed 400 HIV-1 p24 antigen determinations on cell culture supernatants.

Division of Cancer Epidemiology and Genetics

Support Provided by Applied and Developmental Research Program

Applied and Developmental Research Directorate (ADRD) support to the Division of Cancer Epidemiology and Genetics (DCEG) includes both program-dedicated support and support through the Shared Services system.

HPV Immunology Laboratory

- **Infections and Immunoepidemiology Branch** – The Human Papilloma Virus (HPV) Immunology Laboratory provides dedicated support to investigators in DCEG, NCI. Research efforts at the laboratory currently focus on two main areas: (1) immune responses to the HPV vaccines and in the context of natural infection with HPV; and (2) immune/inflammatory markers associated with increased risk of cancer. The laboratory also has a broader interest in developing assays and tools for immune-epidemiological research.

  The laboratory continued to assess HPV antibody responses in individuals with natural HPV infection and vaccine recipients. In addition, as part of the DCEG Initiative on Cancer and Inflammation, the laboratory conducted a number of studies to evaluate the associations between circulating inflammation and immunity markers, and the development of various types of cancers using multiplex Luminex-based bead arrays. In addition, the laboratory continued efforts to validate the use of Luminex technology for analyses of a large number of cytokines, chemokines, and growth factors in bile. Specific achievements are listed below:
- **HPV immunity studies in vaccine and natural infection studies** – It is generally accepted that the efficacy of HPV virus-like particle (VLP) prophylactic vaccines is mediated in large part by neutralizing antibodies against the L1 protein. In addition, it is believed that antibodies produced in the context of natural infection may play a role in protection against subsequent. During this year, the laboratory examined the levels of HPV-16 antibodies in a DCEG and Moffitt Cancer Center collaboration in a cohort of males from Moffitt Cancer Center in a total of 2,638 tests (22,080 wells including standards and controls).
- **Associations between immune/inflammatory markers and cancer risk** – Chronic inflammation and immune alterations are recognized as important etiologic factors for several cancers, but studies of immunity and cancer in the past have been limited in scope and size. A previous study of samples from the Prostate, Lung, Colon, and Ovarian (PLCO) Cancer Screening Trial cohort evaluated the reproducibility of multiplex panels for the measurement of a large number of immune markers (97 different markers). Based on the results of that study, a Luminex-based multiplex immune panel was designed for use in DCEG studies. The HPV Laboratory has evaluated patterns of immune/inflammatory markers associated with the development of several cancer types or infections, including gallbladder, lung, colon, and endometrial cancers, and HPV persistence at the cervix. In addition, the laboratory conducted methodological studies to evaluate kit stability and feasibility of studying a variety of immune markers in bile. A total of about 4,325 specimens were tested for different markers (up to 65 markers) in different panels, with a total of approximately 29,376 wells tested, including QCs and standards. During FY2016, the results from various studies were published. These studies contributed to the identification and understanding of the association between immunity/inflammation and cancer risk in well-characterized cohorts.
- **HPV VLP production** – Because of the large demands for HPV VLPs in ongoing projects, the HPV Immunology Laboratory continued production of a large amount of HPV VLPs (HPV-16 L1 VLPs: 60 mgs and HPV-18 L1 VLPs: 8 mgs). The laboratory also continued the production and validation of various plasmids necessary for HPV VLP production of new HPV types of interest. More than 15,000 wells were also tested for validation and QC and 6,816 wells were tested to evaluate influence of type of blood collection tubes in the field in serum antibody measurements.
- **cCRADA Partnerships** – A collaborative research agreement was executed with Dr. Anna Giuliano at the Moffitt Cancer Center to investigate immune responses at the oral cavity in a clinical trial of mid-adult males vaccinated with the quadrivalent vaccine. A set of 100 sera and saliva samples were tested for HPV-16 and HPV-18 antibody levels by ELISA and 100 serum samples were tested for anti-HPV antibodies following vaccination.

- **LDER Project** – A project on oral immunity in HPV-associated oral cancers was initiated during this fiscal year in collaboration with Dr. Giuliano at the Moffitt Cancer Center. The goal of this project is to identify immune signatures associated with HPV associated oral cancers, using flow cytometry, proteomics, and transcriptomics. Agreements were executed and procedures were established. Feasibility studies were conducted to determine ideal conditions and ideal oral collection procedures. A total of 15 patients and 15 controls were evaluated to immune cell subsets at the oral cavity. In addition, saliva and cell pellets were collected for proteomics and RNA sequencing.

Clinical Support Laboratory

The Clinical Support Laboratory (CSL) provides 0.5 dedicated full-time equivalent staff support to Dr. Margaret Tucker for receipt of blood samples in order to produce and cryopreserve Epstein-Barr virus (EBV)–transformed B cell lines or skin biopsy samples for the generation of primary fibroblast cell lines. A total of 24 blood samples and 21 skin samples were received, with 15 EBV and 11 fibroblast cultures completed. For each sample, the target was to obtain vials from two separate freezes of each sample for long-term storage.

The generation of EBV-transformed B cell lines and primary fibroblasts was also performed through the Shared Services system for other DCEG investigators under YT11-231, as summarized below:

Investigator	EBV (Received/ Completed)*	Primary Fibroblast (Received/ Completed)*
Dr. Sharon Savage	191/83	NA
Dr. Blanche Alter	37/20	12/9
Dr. Doug Stewart	115/12	8/8

*Includes samples received in FY2015 but finished in FY2016.

Biomarker testing support:

- The laboratory performed testing in support of CSAS-17248 from Dr. Charles Rabkin, Viral Epidemiology Branch (VEB), to measure erythropoietin and lactoferrin by ELISA in 110 breast milk samples.
- In support of CSAS-17237 from Drs. Alina Brenner and Martha Linet, Radiation Epidemiology Branch, the laboratory tested 1,800 serum samples in a customized panel of multiplex and singleplex electrochemiluminescence assays. A total of 150 test plates were set up, with a total of 25,200 data points collected.
- In response to CSAS-16764 from Dr. Jonathan Hofmann, Occupational and Environmental Epidemiology Branch (OEEB), the lab tested soluble CD27 and soluble CD30 in 137 serum samples from the Agricultural Health Study.
- In response to CSAS-16797 from the Infections and Immunoepidemiology Branch (IIB), the laboratory performed a four-plex vascular injury panel on 147 serum samples from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study.

Additional support:

- In response to CSAS-16702 from Emily Vogtmann and Dr. Christian Abnet, Nutritional Epidemiology Branch, the laboratory processed approximately 400 saliva samples for repository storage.
- CSL worked with LCMI staff to complete multiple aliquoting projects, which are summarized below:
  - Biostatistics Branch
    - CSAS-15785 = 130 serum aliquots
  - Hormonal and Reproductive Epidemiology Branch
    - CSAS-16954 = 170 serum aliquots
    - CSAS-17245 = 1,086 serum aliquots
    - CSAS-17724 = 330 serum aliquots
  - Occupational and Environmental Epidemiology Branch
    - CSAS-16313 = 1,269 serum aliquots
    - CSAS-17274 = 654 urine aliquots
  - Nutritional Epidemiology Branch
    - CSAS-16946 = 2,112 serum aliquots
    - CSAS-17201 = 4,572 serum aliquots
    - CSAS-17585 = 124 urine aliquots
  - Infections and Immunoepidemiology Branch
    - CSAS-16669 = 100 serum aliquots
    - CSAS-16828 = 28 urine aliquots
    - CSAS-17051 = 18 aliquots from serum and cerebrospinal fluid samples
    - CSAS-17362 = 530 plasma aliquots
  - Clinical Genetics Branch
    - CSAS-17180 = 8,153 cervical sample
  - Viral Epidemiology Branch
    - CSAS-17292 = 789 serum aliquots
  - Genetic Epidemiology Branch
    - CSAS-17172 = 4,950 sputum aliquots

Bioprocessing Laboratory

The BioProcessing Laboratory completed eight projects for DCEG investigators to generate quality assurance (QA)/QC samples for assay development and hormone receptor testing, resulting in the generation of 7,745 aliquots of serum, plasma, and urine. The laboratory also designed and coordinated distribution and returns of donor questionnaires.

Human Genetics Program

Support Provided by the Cancer Genomics Research Laboratory

Over the past year, the Cancer Genomics Research Laboratory (CGR) has continued to function as a high-throughput genomics laboratory in service to NCI's Division of Cancer Epidemiology and Genetics (DCEG). In a highly collaborative endeavor, CGR works closely with investigators to design, plan, and execute a variety of scientific projects. With a focus on automation and cutting-edge technology and innovation, CGR provides comprehensive genomics laboratory and scientific research support from project inception through specimen preparation, data generation, analysis, and publication of findings.

Significant Achievements

Consolidation of CGR and the DNA Extraction and Staging Laboratory (DESL) at the Advanced Technology Center (ATC): The DESL was merged with CGR in November 2011. While CGR was housed at the ATC in Gaithersburg, DESL was located in Building 1066 at the Frederick National Laboratory for Cancer Research (FNLCR) at Fort Detrick in Frederick. Initially, space was not available at the ATC to allow for consolidation of the two laboratory groups. Over the last five years, remaining non-DCEG occupants have been moved from the ATC, opening up space and making it possible to move DESL to the ATC. ATC room 115 underwent minor modifications to house the staging laboratory, while new construction in part of room 109 will generate separate extraction labs for DNA, microbiome samples, and RNA.

While the co-location of these groups has been desired for some time, there has been little impact to operations as a result of being physically separated. At the same time, there are a number of benefits to consolidation, including greater flexibility to move staff between operational teams in order to respond to fluctuating customer needs in different areas, the ability to further develop staff through cross-training, enhanced communication, and group cohesion.

As of this writing, Phase I of the consolidation has been completed. The relocation of the staging laboratory from Building 1066 at the FNLCR to the ATC was completed in May. This represents approximately 85% of DESL's staff, equipment, and materials. Construction of the extraction laboratory at the ATC has begun, and it is anticipated that the extraction lab relocation will be completed by the end of the contract year.

Implementation of Nanoliter-Scale AmpliSeq Library Prep Protocols: Over the past two years, CGR has developed significant capabilities in the area of human papillomavirus (HPV) genome research. Past goals have been focused on the development of HPV whole-genome sequencing assays and application of that assay to over 2,700 HPV+ subjects. This year, the Becton, Dickinson and Company Diagnostics GenCell CLiC LP System was obtained by CGR. This automated liquid-handling instrument has the ability to miniaturize and automate the library prep process used in next-generation sequencing protocols. The process of miniaturizing the library prep reactions will lead to significant cost savings while automation will increase staff efficiency with hands-free operation. Additionally, smaller amounts of input DNA allow samples with limited DNA to potentially qualify for sequencing.

HPV genome research is a priority for DCEG and involves collaboration with a number of investigators within the division. As a result, CGR has built the capability to sequence and characterize HPV in a revolutionary way. Given the impact of this research, it is expected that tens of thousands of samples may be available for characterization. In order to accommodate a scope of this size from both a throughput and cost perspective, development of this automated and miniaturized platform is crucial. Several collaborative proposals submitted to CGR by DCEG investigators will benefit from the increased sample throughput due to the anticipated cost reductions. While currently being used for HPV research, the system will have utility for a variety of other sequencing applications at CGR with similar cost savings over current library prep methods.

Imputation of the Total GWAS Set (TGS): Since 2005, CGR has been a world leader in genome-wide association studies (GWAS). GWAS is defined as screening thousands of individuals utilizing single-nucleotide polymorphism (SNP) genotyping arrays covering polymorphic sites across the entire genome. To date, CGR has genotyped over 150,000 individuals using several generations and versions of arrays. Arrays in the early years started at about 270,000 SNPs, while current arrays can genotype over 5 million SNP sites. In an attempt to pool and normalize array data generated over the last decade, the TGS data set was created by CGR informatics staff and includes data for approximately 118,000 subjects genotyped on one of 14 different arrays. This valuable resource was processed through normalization and cleanup in order to prepare the data for imputation. Imputation is the process of inferring SNPs not genotyped based on data from other samples that do have data at that site. This was computationally challenging for the 118,000 samples currently in the TGS and took approximately five months to complete, but was a necessary step so that data generated in 2005 is equally as valuable as data generated today.

The TGS is now a data-rich resource for DCEG investigators. While the TGS data has been utilized extensively for primary association analyses, there is a wealth of other research that can be conducted as "value-added" studies from this data now that the data set is normalized and imputed. Dozens of proposals have been submitted to receive data from the TGS. CGR is now able to release data to approved users for download for further research.

Validation and Implementation of Next-Generation HPV Typing Assay*:* CGR has developed a low-cost, high-throughput HPV genotyping assay for etiologic and clinical studies.

The initial study used for the typing screen was comprised of 3,500 women with various high- and low-risk HPV types. Each sample has been genotyped using Roche Linear Array (currently one of the most widely accepted HPV typing assays in the clinical space). An initial target of 85–90 percent concordance between the CGR typing assay and the Linear Array methods was targeted, and the results are showing concordance rates exceeding 95 percent.

The HPV typing assay can delineate up to 54 different HPV sub-types including the 13 high-risk strains that account for over 98 percent of cancers associated with HPV. The assay has been designed to be run manually or in automated fashion. The HPV assay was developed using equipment and reagents that facilitate use at laboratories around the world. Current typing assays exceed $100 per sample and are therefore not widely used. The new assay costs less than 25 percent of the currently available assays and represents a crucial advancement in early HPV detection and cancer screening.

Epidemiology and Biostatistics Program

Support Provided by the Clinical Monitoring Research Program

Esophageal Cancer Precursor Lesion Genomic Study – China

Esophageal cancer is the sixth most common fatal human cancer in the world, with more than 406,000 deaths annually, and the fourth most common new cancer in China. More than half of all esophageal cancer deaths in the world occur in China, due to its large population and high rate of this cancer's occurrence. North central China has esophageal cancer rates that are among the highest in the country; nearly all of these cases are esophageal squamous-cell carcinoma (ESCC).

ESCC is an aggressive tumor that is typically diagnosed only after the onset of symptoms, when prognosis is very poor. One promising strategy to reduce ESCC mortality is early detection and a better understanding of molecular pathology and mechanisms underlying esophageal carcinogenesis, which will facilitate the development of biomarkers for early detection.

CMRP staff is working to support the overall esophageal and gastric cancers initiative of the NCI Division of Cancer Epidemiology and Genetics (DCEG). Specifically, support is focused on a cancer precursor lesion genomic study to be conducted in China. The overall objective of this project is to identify and test strategies to reduce mortality from esophageal and gastric cancer in high-risk populations in China. Building on collaborative research that has been ongoing between the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences (CICAMS) and NCI since 1982, the initiative will conduct new studies in this high-risk population to further evaluate etiologic and early-detection hypotheses, and advance disease prevention strategies.

CMRP provides programmatic and project management support for the conduct of NCI's first study under this initiative, titled "Esophageal Squamous Cell Carcinoma Precursor Lesion Genomics Study (Precursor Study)." The goal of this study is to understand the molecular underpinnings of premalignant esophageal lesions. The Precursor Study has two components: (1) a field study to obtain data and biologic samples, which will be conducted by CICAMS, and (2) a genomic analysis to perform sequencing of the biological samples and bioinformatics analysis of the sequencing data, to be conducted by a vendor yet to be determined.

In October 2014, Leidos Biomed CMRP and Research Contracts fully executed Task Order #1 (TO#1) under the Basic Ordering Agreement (BOA) with CICAMS. TO#1 pertains to the conduct of the field study, enrolling a minimum of 150 subjects. Following the execution of the agreement, the Leidos Biomed technical project manager (TPM) met with the NCI PI serving as the main contact for this project to discuss timelines and expectations on deliverables. Per periodic discussions, it became apparent that the Precursor Study would not begin before January 2016 due to the NCI and China protocol review processes. Additionally, although Leidos Biomed was initially requested to establish a second contract to provide sequencing services (sole-sourcing through BGI Americas), the NCI PI informed the Leidos Biomed team that CICAMS is considering directly establishing an agreement with a sequencing vendor (i.e., subcontract through CICAMS) to support the study.

The Precursor Study protocol was approved by the NCI IRB in October 2015 and the CICAMS Ethics Committee (EC) in January 2016. The first patient was enrolled in April 2016. As of July 15, 2016, 20 out of 150 subjects have been enrolled into the study. Patient enrollment into the study has been put on hold to address some concerns raised by the co-investigators. We anticipate enrollment to start up again by the end of August 2016.

Due to the significant delay obtaining IRB/EC approvals for the study protocol, Leidos Biomed and NCI have had ad-hoc meetings only as needed for updates. All pre- and ongoing activities related to the field study are being conducted directly by CICAMS in collaboration with the NCI PI.

On October 28, 2015, Leidos Biomed extended the period of performance of the CICAMS agreement to April 1, 2016 with a no-cost extension. In March 2016, Leidos Biomed fully executed Agreement Modification #3 to: (1) revise the statement of work (SOW), (2) increase the ceiling amount from $180,004 to $210,063, and (3) extend the period of performance to April 1, 2017. The SOW was revised to: (1) restrict the type of esophageal squamous samples collected for analysis to

high-grade dysplasia, and (2) update the timetable for deliverables, per the significant delay with study-start.

The NCI PI anticipates enrollment/sample collection to be completed in one year. Nonseverable funds from two different fiscal years are utilized to support this effort, the first expiring September 30, 2017 and the second on September 30, 2018.

Applied Molecular Pathology Laboratory, Tumor Heterogeneity

The Applied Molecular Pathology Laboratory (AMPL) is a collaboration between the CCR and DCEG to facilitate research using novel, high-throughput techniques for studying tissues in large cancer investigations. The work focuses on handling, processing, and evaluating fixed tissues, with a particular emphasis on using tissue microarrays for immunohistochemical analysis, with subsequent digital imaging and manual or automated evaluation of stains. A physician provides ongoing scientific and pathology support with a focus on: (1) identifying occupational, environmental, and other factors affecting cancer risk; (2) characterizing exposure response relationships; (3) identifying biomarkers for disease detection, diagnosis, and prognosis that are associated with certain risk factors; (4) identifying susceptible populations and gene environment interactions; and (5) improving research methods for investigations. Projects involve sophisticated methods and collaboration among epidemiologists, pathologists, industrial hygienists, and molecular biologists.

The physician provided pathology expertise to various investigators in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, a large, population-based randomized trial evaluating screening programs for these cancers.

Collaboration with the Hormonal and Reproductive Epidemiology Branch, DCEG, and the Pathology and Histotechnology Laboratory, Frederick National Laboratory for Cancer Research (FNLCR): Work continued on the prostate arm of the study using 100 tissue microarray (TMA) slides constructed from prostate cancer tissue and normal prostate tissue from 76,693 men. TMAs had been immunohistochemically stained with antibodies against RAD-9, survivin, Raf-1, and cyclin B1, and then digitalized and analyzed using Aperio Image Scope software. The results of the automated scoring have been reviewed and statistically analyzed. To validate the automated scoring results, the physician performed manual scoring on 100 randomly selected cores for each of the four antibodies. The results of the manual scoring were provided to the investigator.

The assessment of micro-vessel density in breast tissue evaluates the association of this density and mammographic density with breast cancer risk. This study included 465 participants in the Vermont Breast Cancer Surveillance System who underwent an image-guided biopsy to evaluate an abnormality identified on a mammogram. A novel component of this study was the evaluation of global and peri-lesional volumetric breast density. Formalin-fixed, paraffin-embedded (FFPE) tissue was sectioned into whole slides and immunohisto-chemically stained for CD31, a marker of vessel endothelium. The physician developed detailed scoring criteria that were applied to a portion of the slides for a pilot study evaluating the vessel density in normal terminal ductal lobular units and surrounding normal breast tissue in 220 study slides. In total, more than 3,000 scores were obtained by the physician. This work resulted in a manuscript currently in submission for publication.

Collaboration with the Occupational and Environmental Epidemiology Branch, DCEG, and the Pathology and Histotechnology Laboratory, FNLCR: During FY2016, the physician continued to collaborate on two studies within PLCO, analyzing colorectal and bladder cancer slides. The physician reviewed the pathology reports and hematoxylin and eosin-stained tissue sections, identified regions of interest that were selected for TMA construction, and designed the array maps for this project. A manuscript describing the methods and results of PLCO tissue collection is currently in preparation.

Ongoing work for the New England Bladder Cancer Study resulted in one publication in the *J Natl Cancer Inst*.

The CMRP physician initiated three new work efforts during FY2016:

Collaboration with the Radiation Epidemiology Branch, DCEG, and the Pathology and Histotechnology Laboratory, FNLCR: This new work effort aims at evaluating the molecular profile of second primary gastric cancers following treatment for Hodgkin's lymphoma (HL) and testicular cancer to identify possible signatures of radiation- and chemotherapy-induced second stomach cancers. Survivors of HL and testicular cancer have significantly higher rates of stomach cancer than those observed in the general population. The Second GI Cancers Study is an international collaboration to conduct case-control studies to clarify the role of treatment in second primary GI cancers, including stomach cancer. The studies of stomach cancer after HL and testicular cancer showed a clear dose-dependent increase in the risk of stomach cancer following radiotherapy. Among survivors of HL, treatment with the alkylating agent procarbazine was also significantly associated with stomach cancer, but this increased risk was only observed in the presence of radiation doses to the stomach of 25 Gy or higher. It is of great interest to investigate whether there are treatment-related signatures of genomic aberrations in second primary stomach cancers. While recent studies have advanced our understanding about the patterns of genomic aberrations in sporadic gastric tumors, very little is known about second gastric tumors occurring after treatment for a first primary cancer with radiation and chemotherapy. In this collaboration, DNA and RNA will be isolated from FFPE tumor-normal pairs from second primary gastric cancer cases following treatment for HL and testicular cancer to determine whether it is possible to extract sufficient high-quality

DNA and RNA for molecular profiling. Whole-exome sequencing and genome-wide association scans will then be performed to characterize somatic mutations (copy number variation and recurrent mutations) in second primary gastric cancer and to descriptively compare these molecular features across subgroups of cases defined by treatment exposures. The pathologist directs the collaborators in terms of the types, amount, and preparation of tumor and normal tissue to send to NCI for nucleic acid extraction, and optimizes communication with Nationwide on sample preparation/requirements and extraction protocols. The pathologist also reviews tissue blocks and pathology reports of 67 cases of stomach tumors, 24 cases of genetic epidemiology junction tumors, and normal tissue blocks, and annotates areas of interest.

Collaboration with the Clinical Genetics Branch, DCEG, and the Pathology and Histotechnology Laboratory, FNLCR: This study examines the possible unique molecular profiles in second primary breast cancers. The pathologist develops protocols for tissue selection and sample preparation.

Collaboration with the Office of the Director, DCEG, and the Pathology and Histotechnology Laboratory, FNLCR: The B-CAST project includes targeted sequencing of paired tumor- and blood-derived DNA samples. The pathologist develops protocols for tumor sample selection, tissue retrieval, material shipment and processing, and DNA and RNA isolation.

NCI EXTRAMURAL

Division of Cancer Biology

Support Provided by the Data Science and Information Technology Program

Physical Sciences–Oncology Network

This Division of the Cancer Biology Office of Physical Sciences–Oncology brings together cancer biologists, oncologists, and scientists from the fields of physics, mathematics, chemistry, and engineering to address some of the major questions and barriers in cancer research. One of the resources available to this network is a well-characterized set of approximately 40 commonly used cancer cell lines, the Physical Sciences Oncology Network (PSON) cell line panel, that is provided to researchers at the same passage number. All users of the cell lines must follow protocols to ensure that all measurements across all centers are done at the same cell passage number. This design allows a more direct comparison of the results across experiments by carefully controlling the passage number variable.

DSITP has been supporting this office for the last several years by managing projects to characterize physical, genomic, and proteomic properties of the PSON cell line. This year, DSITP continued its support of the PSON cell line proteomic characterization effort. This subcontracted project is providing global and phosphoproteomic characterization data for a subset of the PSON cell line panel.

The goal of these projects is to generate data sets that are publically available to the research community. As part of the closing of the PSON Data Coordinating Center, DSITP was responsible for transferring the PSON cell line genomic and physical characterization data sets to the NCIP HUB (https://nciphub.org/groups/nci_physci/psondcc). This transition ensures that the data remain publically available and also supports the Division of Cancer Biology (DCB)'s goal of transitioning the responsibility for housing these data to the DCB-funded Cancer Systems Biology Consortia DCC grantee.

Cancer Sampling Index (CaSIX)

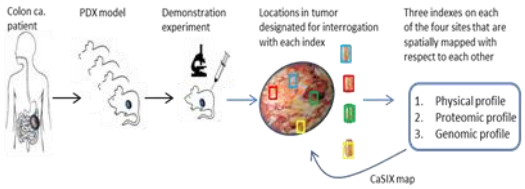
The DCB is supporting the Cancer Sampling Index (CaSIX) pilot project to address the challenges cancer biologists face in understanding the basis of heterogeneity often observed in solid tumors. Relative to many types of cancer, colon cancer is a particularly heterogeneous disease. The nature of this heterogeneity can be a function of the site within the colon from which the tumor arose. For example, colon cancer occurring in the cecum or ascending colon (right-sided tumors) have classifying features that are distinct from cancer originating in descending or sigmoid colon sites (left-sided tumors). Intratumoral heterogeneity can occur at the level of differences in cells comprising a single tumor. Likewise, cellular heterogeneity is a reflection of molecular heterogeneity at the genomic, proteomic, and physical levels.

In the CaSIX project, the spatial geography of heterogeneity as a function of the site of origin within the colon, as well as across an individual tumor, will be assessed through a set of complimentary experimental approaches that measure and co-register genomic, proteomic, and physical parameters. These data would serve to demonstrate the level of heterogeneity within individual tumors and compare those data between tumors that arose in either the right or left colon.

Studies using PDX models of human colon cancer suggest that many features of the primary disease are maintained following passage in mice. PDX models permit a wider range of experimental approaches to measure and map tumor heterogeneity than what would be possible in a patient with colon cancer. The CaSIX project seeks to provide a spatial delineation of heterogeneity in colon cancer at two levels. The molecular to cellular level will link together genomic and proteomic data with physical characteristics at and across multiple sites within an individual tumor. The second level will entail a comparison of these data obtained from multiple PDX models of human colon cancer that have been derived from tumors originating at distinct geographic locations within the colon (e.g., right and left colon).

The utility of the PDX model system to test specific hypotheses on tumor heterogeneity will be elucidated as well as a demonstration of the important project

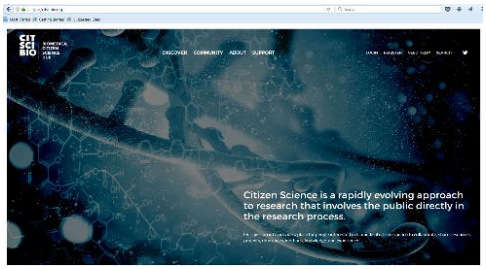
coordination considerations in the collection of spatially co-registered data at the genomic, proteomic, and physical scales.



***Hypothetical CaSIX example**. The spatial geography of heterogeneity across an individual tumor (resected from the descending colon in this case) is assessed through a set of complimentary experimental approaches that measure and co-register genomic, proteomic, and physical parameters from samples obtained at multiple distinct locations within the tumor. The integration of these multiscale data sets will form the Cancer Sampling Index (CaSIX) map.*

Biomedical Citizen Science Hub

The Biomedical Citizen Science Hub is sponsored by the DCB and the Division of Cancer Control and Population Sciences (DCCPS). The goal of the project is to create an online collaboration space for the growing and virtually dispersed biomedical citizen science resources, projects, references, methods, and communities to be discovered and engaged by interested stakeholders.



Launched this year, the CitSciBio.org Hub (https://citscibio.org/), based on the HubZero platform, is a resource for research, education, and collaboration in biomedical citizen science and crowdsourcing.

Support Provided by the Laboratory Animal Sciences Program

NCI Mouse Repository

Mouse models of human cancer have had a profound impact on our current understanding of the mechanisms of tumorigenesis and the pathways regulated by cancer-related genes. These models hold the promise of serving as critical tools in the discovery and testing of novel therapeutics to be used in the treatment and prevention of cancer.

The NCI Mouse Repository is a resource funded by the NCI Division of Cancer Biology for maintenance and distribution of mouse models and associated strains. The repository, which is managed by LASP, makes strains available to all members of the scientific community (academic, nonprofit, commercial). Beginning in November 2015, the NCI Mouse Repository discontinued the maintenance of live animals. Currently, strains are cryoarchived and distributed solely as frozen germplasm.

Significant Achievements

The repository currently maintains 137 strains as cryoarchived stock. An additional 9 strains accepted during FY2016 are at various stages of importation. During the reporting period, 154 orders for live, reconstituted and cryoarchived mice were filled. In total, 335 live animals and 58 embryo shipments were distributed worldwide.

In an effort to address the function that miRNAs play in human cancer, their use as diagnostic tools, and their potential role as new targets for therapeutic intervention in the treatment of cancers, NCI's Division of Cancer Biology is supporting the generation of mES cells engineered to express 500 known mouse miRNAs. The NCI Mouse Repository was charged with making this new, important resource available to the cancer research community. The entire collection was made available for distribution to the scientific community in July 2013. Information regarding this resource is available on the NCI Mouse Repository website (http://mouserepository.cancer.gov). The website also includes validation documentation of the mES cells generated and sequencing data for each miRNA. A manual including all protocols utilized in the generation, care, manipulation, and use of the available mES clones is also provided.

During the reporting period, nine orders for mES cell lines were processed.

Support Provided by the Cancer Research Technology Program

The Antibody Characterization Laboratory (ACL) continues to produce antibodies (approximately 390 to date) and qualifying data as requested by the Office of Cancer Clinical Proteomics Research. There are presently 140 projects in production. Our current additional focus is to generate antibodies for the specialized application in i-multiple reaction monitoring (MRM) assays for both the RAS program and external groups. A significant effort was undertaken to demonstrate that results from the ACL were in agreement with the Paulovich lab. The ACL screened more than 300 candidate supernatants by LC MS/MS in parallel with the Paulovich lab and showed both agreement and selection priority despite using different instrumentation. We also performed a pilot to demonstrate initial screening could be done using a less labor-intensive method, matrix assisted laser desorption ionization (MALDI). In order to demonstrate potential clinical utility, we have acquired an FDA notified triple quad instrument to verify the assays tested on a clinical grade instrument. All antibody and assay data have been uploaded on the Clinical Proteomic Tumor Analysis Consortium (CPTAC) portals, and antibodies are available from the University of Iowa.

Division of Cancer Control and Population Sciences

Behavioral Research Program

Support Provided by the Clinical Monitoring Research Program

The goal of the Behavioral Research Program (BRP), located within the NCI Division of Cancer Control and Population Science (DCCPS), is to increase the breadth, depth, and quality of behavioral research in cancer prevention and control. To this end, the BRP initiates, supports, and evaluates a comprehensive program of research, ranging from basic behavioral research to the development, testing, and dissemination of interventions in areas such as tobacco use, screening, dietary behavior, and sun protection. Over the past 15 years, CMRP has assisted DCCPS in its mission by providing programmatic and scientific support services to all branches within the DCCPS BRP.

CMRP staff support the BRP through a wide range of high-level activities, including: conducting original research on health behavior and cancer; creating new professional development opportunities for BRP and DCCPS staff and fellows; supporting scientific content, communications, and logistics for major meetings, conferences, and symposia; developing and reviewing scholarly publications, including articles and books; and conceptualizing, developing, and maintaining content on public-facing BRP websites.

In FY2016, the senior behavioral scientist, working with NCI colleagues, obtained informal agreement from Kaiser Northern California and Hawaii to participate as a collaborator in a physician survey that will look at how attitudes and knowledge impact the physicians' willingness to recommend cancer treatment clinical trials to patients. Oncologists and surgeons at Kaiser Northern California and Hawaii will be sent the NCI-created survey instrument via Kaiser leadership. We anticipate that by the end of FY2016, this agreement will be formalized and the survey will be completed.

The senior behavioral scientist and colleagues in the BRP co-authored one journal article for *Transl Behav Med* that is expected to be published by the end of FY2016.

Throughout FY2016, the senior behavioral scientist has provided assistance, based on her expertise in team science, to colleagues leading a collaboration between NCI and the American Society of Clinical Oncology (ASCO) to draw attention to the relevance of team research to enhancing coordination of cancer care. She was an invited guest speaker at the NCI-ASCO Workshop on Teams in Cancer Care in Phoenix, AZ, on February 25, 2016. She also reviewed multiple manuscripts for an NCI-ASCO special issue of the *J Oncol Pract* highlighting teams in cancer care, which will be published in late 2016. Her contributions will be cited in the special issue.

Science of Research and Technology Branch

The Science of Research and Technology Branch (SRTB) is located within BRP, NCI DCCPS. The SRTB supports the development and application of innovative research approaches, theories, methods, measures, analytic tools, and technologies that advance social and behavioral science as they relate to cancer prevention and control.

The CMRP behavioral scientist supports key SRTB activities, particularly the work of the branch's Science of Team Science (SciTS) team. The SciTS team is charged with developing the knowledge base on effective and efficient team collaboration in science, and facilitating the growth of the SciTS field, a rapidly growing interdisciplinary area of research dedicated to building collective knowledge for fostering maximal effectiveness in team science. NCI has been a national leader in funding team science, with the aim of accelerating innovation and progress around scientific priority areas.

During FY2016, the behavioral scientist gave six invited talks at scholarly venues including workshops, conferences and graduate courses and co-authored an invited book chapter, a peer-review journal article, and a conference poster. Additionally, the behavioral scientist worked as an editor with co-editors from NCI SciTS and DCCPS for a book with the working title "Advancing Social and Behavioral Health Research through Cross-disciplinary Team Science: Principles for Success." In the role of editor, the behavioral scientist identified authors for each chapter, obtained commitments from authors, and worked with the NCI co-editors to review all submitted material and draft chapters. It is anticipated that Springer, Inc. will publish the book in FY2017.

The behavioral scientist continued to co-lead the enhancement, dissemination, and evaluation of the Team Science Toolkit—www.teamsciencetoolkit.cancer.gov—an online, one-stop-shop for resources for using, facilitating, supporting, evaluating, or studying team-based collaboration in science. This effort included a successful communications and dissemination campaign for the Toolkit, ensuring that the Toolkit continues to be featured on the NIH homepage, the Office of Behavioral and Social Science Research (OBSSR) homepage, and on the SciTSlist listserv maintained on behalf of NCI. A second sync of the Toolkit website with the Mendeley Team Science user group was conducted to ensure that the Toolkit leverages the bibliography present in the Mendeley group. The behavioral scientist continues to identify and cultivate bloggers for the Toolkit website, and shepherd blogs through the process of drafting, editing, and publishing on the Toolkit website. The bloggers have self-reported that their blogs have helped to advance the legitimacy of their work in the SciTS field, at their institutions, and elsewhere, by serving as a forum to share scholarly writing in SciTS topics that may be hard to publish in the current range of journals, as currently there is no journal dedicated to SciTS.

During FY2016, the behavioral scientist served on the Planning Committee for the SciTS 2016 conference. In this capacity, she contributed to identifying key scientific conference themes and speakers and disseminating the call for abstracts. She also served as a peer reviewer for submitted abstracts, grouped accepted paper and poster abstracts into thematic sessions, recruited moderators for all paper sessions, facilitated a lunchtime roundtable discussion on SciTS research and evaluation, and developed an online survey delivered daily to all conference attendees and the SciTSlist. The survey included key questions about the future of the SciTS community to engage the range of stakeholders in this discussion. She also participated in a series of planning meetings for the 2017 conference and the future of the SciTS field.

The behavioral scientist continues to serve in an expert advisory capacity to support colleagues at NCI and in related groups. The Transdisciplinary Research on Energetics and Cancer (TREC) 2 initiative is a major funding initiative of NCI that supports four research centers and a coordination center to conduct team-based transdisciplinary research to examine the relationship between obesity and cancer. As a member of the TREC 2 Collaboration and Outcomes Working Group, the senior behavioral scientist provided expert advice to members of the TREC 2 coordination center to support their work in evaluating processes and outcomes of transdisciplinary team science in TREC 2 research centers. She helped them conceptualize and design research studies and shared with them interview guides and coding schemes. She has also provided expert advice and assistance about SciTS, team science, and evaluation research to colleagues at the NCI and NIH more broadly. For example, she advised members of the NIH Office of Disease Prevention on best practices for facilitating collaborations across institutions and disciplines.

The behavioral scientist also leveraged use of undergraduate level interns to support SRTB programmatic and administrative activities in 2016. These unpaid interns work for 40 hours per week and engage in such activities as: helping to develop PowerPoint slides for SRTB staff members, developing and managing spreadsheets to track the flow of chapter submissions and reviews for the book, "Advancing Social and Behavioral Health Research through Cross-disciplinary Team Science: Principles for Success," creating Endnote libraries, and attending to other administrative and project support activities.

The behavioral scientist's support will be ending with the conclusion of FY2016.

Basic Biobehavioral and Psychological Sciences Research Branch

The mission of the Basic Biobehavioral and Psychological Sciences Branch (BBPSB) is to advance research in biobehavioral mechanisms and psychological processes to reduce cancer risk and improve outcomes. The BBPSB research agenda includes, but is not limited to: (1) basic mechanisms of cognition, emotion, judgment, and decision making; (2) biological mechanisms of psychosocial influences on cancer biology and outcomes; (3) methodology and measurement of basic psychological, cognitive, and affective processes; (4) biobehavioral mechanisms of co-morbidities associated with cancer and cancer treatment; (5) basic mechanisms of sensation, attention, and perception as related to cancer risk and control; and (6) basic mechanisms of the placebo effect.

CMRP staff provides scientific, programmatic, and administrative support and travel coordination, and establishes consulting agreements and research subcontracts for the BBPSB. In particular, CMRP staff is part of the Network on Biobehavioral Pathways in Cancer Scientific Management Committee (SMC) and provides guidance and support to network initiatives and research projects.

A clinical project manager provides support to BBPSB initiatives as well as other DCCPS initiatives as a whole. The clinical project manager, working with the BBPSB chief, assists with developing Requests for Proposal (RFPs) for new research initiatives, as well as maintains and provides oversight for awarded research subcontracts. Three existing research subcontracts continued in FY2016: (1) a subcontract with the Medical College of Wisconsin to assess whether gene expression of beta-adrenergic signaling pathways can be altered in individuals undergoing autologous hematopoietic stem cell transplantation for multiple myeloma by administering a daily beta blocker (propranolol); (2) a subcontract with Rutgers Robert Wood Johnson Medical School (which concluded in December 2015) to investigate the hypothesis that chronic stress promotes DNA damage and enhances mutation frequency and copy number variation in Apcmin/+ mice in a largely p53-dependent manner; and (3) a subcontract with Massachusetts General Hospital (which concluded in February, FY2016) to investigate potential support for an association between oncologists' emotional state, including burnout, and their practices for administering chemotherapy to patients with metastatic cancer at the end of life.

In 2016, BBPSB decided to continue the Washington University in St. Louis project, expanding the scope of work. The original work provided a systematic review of the representation of individuals with multiple chronic conditions in randomized, controlled trials of behavioral and psychosocial interventions published in general medical, behavioral medicine, behavioral science, health psychology, social science, and public health journals. In early FY2016, CMRP staff modified the existing

agreement to include the development of a methods document, to describe, in detail, the methods of the systematic review, including categories and coding schemes that were developed to adequately describe the inclusion and reporting of the inclusion of multi-morbidities in published clinical trials. This document will provide a framework for future investigations. In March 2016, the agreement was again modified to support efforts to (1) transform the existing database into an accessible form by cleaning the data and producing additional documentation, and (2) produce a more focused review of the existing database, examining eligibility criteria and the inclusion of multi-morbidities specifically in trials with cancer survivors. These new efforts are expected to continue into FY2017.

In early FY2016, CMRP staff awarded two new agreements resulting from RFPs released in FY2015. The first agreement, awarded to Monash University, supports a pilot study to assess the impact of beta blockade on gene expression in early stage breast cancer. The second, awarded to Columbia University, seeks to support a multistage planning effort for single-subject trial design that incorporates sensor and mHealth technologies to address the problem of patient-by-treatment interaction in the context of depression symptom management, cancer survivorship, and multi-morbidity. The clinical project manager assisted the subcontracts department with the award process, developed progress report templates and communication plans, worked with the vendors to develop deliverable schedules, arranged in-person meetings with NCI and vendor program staff as required, and hosted the project kick-off calls for these new agreements.

Also in FY2016, CMRP staff worked with the BBPSB program chief to award a sole-source Phase II project to the University of California, Los Angeles, to continue work begun under a previous agreement. The project seeks to validate several functional magnetic resonance imaging (fMRI) probe tasks that may be used to help better understand some of the relationships between social support, stress, and cancer progression. The current work will be conducted in a sample of breast cancer survivors. The clinical project manager assisted with developing the SOW, convening the Source Evaluation Group (SEG) to evaluate the proposal submitted in response to the RFP, and worked with the subcontracts administer to award the agreement.

CMRP staff, in conjunction with the CGH conference team, worked with the BBPSB program chief to host a capstone meeting for the Network on Biobehavioral Pathways in Cancer to showcase the network's accomplishments over its five-year period of support. CMRP staff provided planning and logistics support, including inviting and tracking attendees, developing timelines, and providing meeting materials and onsite support.

Health Behaviors Research Branch

The mission of the Health Behaviors Research Branch (HBRB) is to support research on cancer prevention behaviors and outcomes, including diet, physical activity, sedentary behavior, energy balance, obesity, sun safety and indoor tanning, genetic influences on behaviors, and virus exposure. Activities include providing leadership in developing methodologies for measuring health behaviors and psychosocial correlates of behaviors, focusing research on effective multilevel influences, examining the interaction between the environment and psychosocial factors, evaluating interventions and policies, and promoting training and dissemination of information related to behavioral health research. The CMRP senior behavioral scientist supports three key initiatives within HBRB: the Family Life, Activity, Sun, Health, and Eating (FLASHE) Study, the Classification of Laws Associated with School Students (C.L.A.S.S.) website and database, and systems science modeling and education.

The FLASHE Study seeks to examine psychosocial, generational, and environmental correlates of cancer-preventive behaviors in a one-time observational survey. The goal of this survey is to advance the understanding of the dynamic relationship between the environment, psychosocial factors, and behavior from an intergenerational perspective (i.e., assessing adolescent–parent dyads). The senior behavioral scientist serves as the FLASHE Study director, overseeing daily management, subcontract implementation, and study protocols, and has been instrumental in navigating the government customer through the strengths, challenges, and limitations of systems modeling in behavioral research. The senior behavioral scientist received an NCI Director's Award for developing this study.

The FLASHE Study closed its data collection period effective October 2014. Since that time, the senior behavioral scientist has provided oversight on all aspects of data management and preparation of the FLASHE data for public release. Almost all materials have been prepared for public release, and relevant updates to the FLASHE webpage began in spring 2016 (methods report, dyadic analysis workshop video, etc.). Data release launched in June 2016. All FLASHE survey data will be publicly available before the end of the fiscal year.

The senior behavioral scientist conceptualized a special journal supplement focused on the FLASHE Study, which includes seven to eight key papers on FLASHE methodology. This supplement will be published in the *Am J Prev Med* (a premier public health journal with an impact factor of greater than four) and will serve as a way to promote the use of FLASHE data among the extramural community and provide a key reference source for data usage. All manuscripts were submitted, and the issue is expected to be published by mid-2017.

The journal supplement represents a cost-savings initiative. The senior behavioral scientist corresponded and requested quotes from several journals, identifying a journal that would offer the greatest flexibility, combined with rigor and visibility, and balanced by cost. Publishing each

manuscript individually could have taken much longer and potentially had higher costs depending on publishing success.

The senior behavioral scientist created and executed a workshop on dyadic analysis as part of the subcontract to two extramural scientists. This event, held in February 2016, had over 250 registrants and is now successfully archived on the NCI FLASHE website and serves as a learning resource for NCI staff and the public.

Epidemiology and Genomics Research Program

Support Provided by the Clinical Monitoring Research Program

The Epidemiology and Genomics Research Program (EGRP) funds research in human populations to understand determinants of cancer occurrence and outcomes. The program fosters interdisciplinary collaborations and the development and use of resources and technologies to advance cancer research and its translation to the basis for clinical and public health interventions. EGRP's aim is to reduce the cancer burden by identifying determinants of cancer risks and improving outcomes.

The National Human Genome Research Institute (NHGRI) has partnered with NCI to fund efforts to gather evidence on clinically relevant genetic variants, identified through whole-genome sequencing (WGS). The effort currently referred to as the Clinical Genome Resource (ClinGen), formerly called the Clinically Relevant Variants Resource (CRVR), requires detailed, systematic evidence about each variant that is being considered for clinical relevance.

CMRP staff awarded a research subcontract to Kaiser Permanente in March of FY2014 to support this effort. The project objectives were to develop an evidence synthesis protocol for each proposed gene/phenotype pair, produce evidence-based reports (case studies), perform semi-quantitative assessments of the clinical actionability of gene/phenotype pairs, and coordinate with the other groups of the ClinGen.

EGRP wished to continue this effort in FY2016. CMRP staff worked with NCI staff to develop an SOW and solicited a proposal from the vendor. The proposal was accepted and work on Phase III of the project began in FY2016.

In addition to Phase III, a need was identified for the development of an automated tracking system to track the volume of topics and related assignments through the various stages of development, review, revision, and scoring. The new tracking system enables functionality such as adding new topics to the list for review, tracking topics' ongoing and changing status, indicating the personnel assigned to the topics at various phases, and generating alerts to staff when topics are near deadline, past deadline, or complete. A modification to the agreement was executed in FY2015 to include development of the tracking system, which continued into FY2016. Work on the tracking system concluded in January 2016, ahead of schedule, and allowed more time to use the tracking system during Phase III.

EGRP decided to continue work on this project in FY2017. CMRP staff worked with EGRP to develop a statement of work for the effort and solicit a sole source proposal from the vendor for evaluation. Phase IV of the project is expected to begin at the start of FY2017.

Division of Cancer Prevention

Support Provided by the Applied and Developmental Research Directorate

The BioProcessing Laboratory provided support to Division of Cancer Prevention (DCP) Phase III clinical trials for specimen processing, repository management, specimen data management, site assessments, and meeting support. These studies are the Selenium and Vitamin E Cancer Prevention Study (SELECT), Prostate Cancer Prevention Trial (PCPT), Prostate, Lung, Colon, and Ovarian Cancer Trial (PLCO), and the 2003 DCP Consortia for Early Phase Prevention Trials. Additionally, the laboratory provided collection acquisition support for the centralization of existing collections of DCP's Early Detection Research Network, conducting site, data, and collection assessments for two clinical trials, one of which will physically relocate in fiscal year 2017. This same type of assessment was conducted on behalf of the DCP for the relocation of the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 study, which will physically relocate in fiscal year 2017.

Biospecimen Processing and Repository Management

Study	Material	# Handled (sum of extracted, distributed, sent to storage)	Activity
SELECT	DNA	1,280	Extract, quantify, normalize, aliquot, and store.
EDRN	Multiple	66,783	Organize logistics for the acquisition of specimens in their freezers stored at another facility, and coordinated the data mapping to the Biospecimen Inventory Management (BSI) database.
PLCO	DNA	16,018	Extract, quantify, normalize, aliquot, and store; distribute aliquot to extramural investigator.
PLCO	Serum	15,138	Aliquot, store; ship subset to investigator.
PLCO	DNA	16,674	Received for re-inventory, relabeling, and re-quantification.

Support Provided by the Clinical Monitoring Research Program

Central Institutional Review Board (CIRB)

NCI's Central Institutional Review Board (CIRB) initiative was implemented in 2001 to reduce the administrative burden on local IRBs and ensure the human research protection of NCI's clinical trial participants. In 2015, the Cancer Prevention and Control (CPC) CIRB was added. This board reviews clinical trials funded through the Division of Cancer Prevention (DCP). DCP needed to acquire the specialized expertise to perform regulatory oversight on this complex, highly visible, and highly sensitive project, enabling it to respond quickly and correctly to issues as they arise to ensure the long-standing integrity of the NCI CIRB initiative.

In July 2015, CMRP began to manage a consulting agreement with Lisa Rooney, an expert in human subjects protection. Ms. Rooney has expert knowledge of federal regulations and extensive experience in regulatory compliance as it applies to federally funded clinical trials conducted in a large, complex organization across a variety of cohorts and trial designs. She independently provides the services of a human subjects research expert in order to satisfy the overall operational objectives of NCI's DCP.

At the beginning of FY2016, Leidos Biomed exercised agreement Option Period 1, increasing funding and extending the period of performance for this agreement. Option Period 2 was exercised in summer 2016, extending the agreement through September 2016.

During this reporting period, Ms. Rooney reviewed and provided feedback on multiple CIRB protocols; developed a "red flag" concept paper, which included reviews of several studies; presented at the DCP CIRB education day titled "IRB Approval Criteria IRB Decisions," presented "NPRM: Revising the Common Rule" at the October 15, 2015 CIRB Operations meeting, and presented an analysis of a French study "The BIA 10-2474 Study: What Went Wrong?"; developed the "IRB Approval Criteria and Stipulation Assessment Tool," and attended various CIRB meetings. The CMRP PMO continues to provide support for this agreement.

Division of Cancer Treatment and Diagnosis

Office of the Director

Support Provided by the Applied and Developmental Research Directorate

Clinical Pharmacodynamics – Biomarkers Program

As part of the reorganization and consolidation of support to the Division of Cancer Treatment and Diagnosis (DCTD), the Phase I/II Sample Processing Laboratory, the Preclinical Assay Development and Implementation Section (PADIS), and the National Clinical Target Validation Laboratory (NCTVL) were grouped together under the umbrella of Clinical Pharmacodynamics – Biomarkers Program.

Preclinical Assay Development and Implementation Section

Assay development, transfer, and preclinical support

- Developed and validated a multiplex immuno-fluorescence assay that quantifies the levels of epithelial-mesenchymal transition (EMT) biomarkers (E-Cadherin, Vimentin, β-catenin) in formalin-fixed, paraffin-embedded (FFPE) tumor tissues (EMT-IFA). FFPE human tumor xenografts and cell lines serve as calibrators and reference materials for establishing image acquisition parameters for segmented tumor regions of interest. Using EMT-IFA, PADIS discovered that pharmacological targeting of VEGFR/FGFR/PDGFR signaling with the multi-kinase inhibitor pazopanib stimulated EMT in a preclinical xenograft model of gastric carcinoma (MKN45), as revealed by the significant increase in the V:E ratio (P=0.0023) coupled with increases in the

EMT phenotype (co-localized V+E+ at the individual cell level) (P=0.0070). Over 210 specimens from five xenograft models were analyzed using this assay.

- PADIS is developing multiplex panel(s) to survey the activities of the signaling pathways through Erk, MEK, mTORC, AKT, and PI3K (as modified by PTEN) in tumor biopsies of clinical disease as well as preclinical models in order to discover the signals of drug action or drug reaction that will be the most informative pharmacodynamics (PD) biomarker. Development of multiplex requires a number of novel, high quality monoclonal antibodies that recognize specific isoforms of signaling molecules, or post-translational modifications in these molecules that indicate their activities and are modulated by drugs. PADIS successfully developed highly selective monoclonals to MEK1 and MEK2 isoforms, validated them using knock-out cell lines, and demonstrated their applicability to a multiplex Luminex platform. In addition, PADIS produced recombinant MEK1, MEK2, ERK1, ERK2, and three mutant forms of ERK/MEK proteins as calibrators.
- PD analysis of preclinical studies to support DCTD drug development: Completed fractionated extraction (cytosol and membrane fractions) of over 600 preclinical xenograft tumor samples. Also completed 14 biomarker multiplex apoptosis panel analyses of more than 1,000 lysate samples to generate and analyze approximately 15,000 biomarker data points, primarily using the apoptosis multiplex assay.
- Transfer of the multiplex EMT-IFA to assess the EMT in tumors is in transfer to the NCTVL, with completion anticipated in September 2016. Ninety-six assays have been performed on specimens from 16 patients to date with this new assay.
- Transfer of the DDR2 multiplex assay for analysis of PD biomarkers on tissue specimens to NCTVL was initiated.
- In support of the above two assays, substantial internal and extramural software development was performed, then validated, to include the following:
- Optimized DDR2 slide based imaging on the Nikon confocal platform to perform all imaging (including widefield capture) in a single run speeding up acquisition over 33 percent.
- Developed multichannel image registration, alignment, and merging to work with the optimized DDR2 acquisition.
- Developed Definiens methods for the extraction of the plasma membrane, cytoplasm, and nuclear spaces in a single script. Applicable for MET/pMET analysis and other membrane associated markers.
- Developed and validated an assay for autophagy, employing the biomarker LC3, for use on paraffin embedded sections and subsequently applied to clinical specimens in a research test, as part of demonstration of clinical readiness, for the COTC007 clinical trial.
- Developed and validated an assay analysis method for coexpression of γH2Ax+ cleaved Caspase 3 as an apoptosis method for FFPE sections and applied it to analysis of clinical specimens for the COTC trial.
- PADIS supported the DCTD unexpected drug synergy program. This program has moved three drug combinations discovered as active in the American Recovery and Reinvestment Act of 2009 (ARRA) program into preclinical development, with the objective of filing IND applications and initiating clinical trials. PADIS developed evidence for the mechanism of action and provided a mechanistic biomarker to be employed in monitoring drug activity.
    - Drug combination of nilotinib (50–100 mg/kg, QDx5, PO) was tested with paclitaxel (10 mg/kg, Q4Dx2, IV) [abbreviated N+T] in a breast cancer MDA-MB-468 model and with docetaxel (7.5–15 mg/kg, Q4Dx2, IV) in a glioma U251 model. IFA analyses of xenografts confirmed pHH3 activation consistent with the known mechanism of action(MOA) of paclitaxel. Over 300 specimens were processed for this project.
    - Drug combination (N+T) demonstrated regression in tumor volume in responsive models. A survey of N+T treated tumors in the U251 model showed reduced levels of Mcl1—BAK heterodimer, which was accompanied by increased mitochondrial BAX and cytosolic cleaved caspase-3 levels. Validation of biomarkers for the N+T combination is underway.
    - The N+T drug combination also showed dramatic induction of the EMT state and evidence of selective cell killing that did not include the transitional cell population. Additional fit for purpose analysis of the EMT-IFA was performed on MDA-MB-468 triple negative breast xenografts treated with vehicle, nilotinib alone, paclitaxel alone, or the combination for 18 days followed by the removal of the drug at D18, then reanalysis at D57. By EMT-IFA, the drug combination was found to efficiently reduce the percent of E+ cells in tumor tissues, resulting in a 90 percent reduction of tumor cells at D18. The majority of the remaining cells were mesenchymal or V+. The total percent of transitional tumor cells within V/E co-localized areas remained the same. When combo treatment was terminated at D18, tumor cells regrew, resulting mostly in epithelial or E+ tumors cells at D57. Thus, the EMT-IFA showed that epithelial tumor cells showed greater sensitivity to the drug combination treatment compared to single agents and that the transitional and mesenchymal cells were more resistant to combination therapy.

- Immuno-PDs: PADIS initiated work on a program to identify PD and mechanistic markers of immune modulator therapies focused on identifying and validating key reagents and in vitro–mixed culture systems.
- Showed feasible application of a PD1 monoclonal antibody for IHC staining on activated T-cell lymphocytes stimulated by anti-CD3, -CD28 antibodies after 7 days. Also showed feasible application of a SHP-2 pY542 monoclonal antibody for IHC staining of activated T lymphocytes stimulated by anti-CD3, -CD28 and -CD137 bead stimulation after 7 days.
- These reagents are feasible candidates for IFA multiplex development on tumor biopsy samples.
- Demonstrated that Keytruda (Pembrolizumab), the therapeutic antibody to PD-1, was able to bind recombinant PD1 by Western and ELISA. Keytruda was shown to compete and displace the binding of recombinant PDL-1-FC to PD1-His by ELISA. The reverse, however, was shown not to be true; PDL-1 FC cannot compete with Keytruda for binding to PD-1 His by ELISA.
- Tested various canine phenotypic and agonistic CD antibody markers for staining canine PBMCs (CD3, CD8, CD5, CD28, CD45R) by flow analysis.
- DNA Repair Pathway Activation: In-house development has continued on multiplex assays to survey DNA repair pathway activation on pathology slides from tissue biopsies. The second multiplex of four DNA repair markers (phosphoS343Nb1s, γH2AX, pATR, and RAD51) have completed fitness-for-purpose testing in a mouse xenograft model and clinical readiness testing. Assay SOPs and calibrators were completed and assay transfer to NCTVL was initiated in a modified form since NCTVL does not have the equipment to acquire and analyze focal image data from cell nuclei. Over 200 assays were performed on clinical specimens with this multiplex and over 300 preclinical specimens were also analyzed.

Clinical trials support

Clinical trials support continues to be a significant part of PADIS' activities; 19 clinical trials with patient specimens sent from 16 extramural sites plus the Developmental Therapeutics Clinic/DCTD were supported. PADIS performs assays that cannot be readily transferred to other laboratories due to equipment availability (e.g., CellSearch® and Confocal Multiplexed Microscopy) or assays that are highly exploratory and require additional evidence of clinical utility before dissemination to the wider community. Specific accomplishments are outlined below.

- PADIS provided ongoing clinical trial support; >205 patient biopsy specimens were received, processed to block, and assayed under either the DDR2 and/or EMT multiplex protocols (including 9483, 9510, COTC007b, 8273, 8880, 9350).
- PADIS received and processed >292 blood specimens for PD testing on the Apostream instrument and >112 blood specimens for PD testing on the 5 channel CellSearch system.
- PADIS continued direct support of the NCI Clinical Center trial of the indenoisoquinolines with quantitative, validated assays of γH2AX for PD in circulating tumor cells (CTCs) for the P8273 and the COTC007b clinical trials.
- Analysis of CTC specimens from the FdC+THU trials P8351 and P9127, and the TdC trial P9883 confirmed that re-expression of P16 in treated patients is a valid pharmacodynamic marker of drug activity that correlates with disease stabilization in patients.
- PADIS completed analysis of clinical biopsy specimens demonstrated induction of EMT in treated patients. Analysis of CTC specimens from this trial confirmed presence of the transitional epithelial + mesenchymal phenotype cells in patient blood, providing evidence that measurements of biomarkers in CTCs correlate with similar measurements in patient biopsy specimens.
- Clinical Trial CTEP#8880: Pazopanib and ARQ 197 for Advanced Solid Tumors (NCT01468922). PADIS completed the primary endpoint analysis of total MET and pMET biomarkers post pazopanib treatment. Paired biopsies were evaluated in 8 pts, and levels of full length MET (total) were measurable in 6 biopsy pairs, ranging from 3.1 - 626.3 fmol/mg protein. Pazopanib treatment decreased intact MET levels in 5 out of 6 pts; similarly, it decreased pMET levels in 3 of 3 pts. These data established clinical utility of MET and pMET assays that were developed and validated at PADIS. Contrary to the trial hypothesis that blockade of VEGFR would result in activation of MET, our results revealed that pazopanib treatment resulted in decline in MET levels and/or pMET levels. These findings were presented at ASCO 2016.
- PADIS implemented a Specimen Quality Tracking System and reorganized all physical specimen banks to enable continuous improvement tracking and to assist the clinical community in improving the overall quality and usability of biopsy specimens collected during clinical trials.

- Other clinical trials supported are listed in the table below:

Clinical Trial	Clinical Center	Investigational Agent
P7002	PDX	
P8273	DTC	Indenos
P8351	DTC/UPMC / COH	FdC+THU Phase II
P8484	CTEP/Farber	888 + SCH727965 +/- Carboplatin
P8811	CTEP/COH	888 + Carbo + Paclitaxel
P8875	DTC	Sunitinib + Cedirinib
P8880	DTC	Pazopanib + ARQ197 (tivantinib)
P9127	DTC/COH	FdC+THU Oral
P9284	DTC	Cabozantanib
P9350	DTC	MK1775
P9430	DTC	LMP400
P9483	DTC	TRC102+TMZ
P9510	DTC	BMN673
P9605	DTC	Ganetespib+Zif-Afibercept
P9659	DTC	Nilotinib+Paclitaxel
P9762	DTC	Bortezomib + Clofarabine
P9771	DTC	Vx970 + Cisplatin + Veliparib
P9883	DTC	TdCyd
COTC007	DCTD	Indenoisoquinolines

Preclinical drug development support of NExT Program agents

These are ongoing efforts for evaluating the pharmacodynamic activity of new compounds, employing assays already developed and validated by PADIS to support the preclinical development of agents for eventual evaluation in the Developmental Therapeutics Clinic (DTC).

- PADIS support of preclinical development for the TdCyd program continued, including xenograft experiments that compared the activity and biomarker modulation of 4 nucleoside analog drugs including Decitabine, 5-Fluorodeoxcytidine±, Tetrahydrouridine, Azathiodeoxycitidine and Thiodeoxycytidine. 430 specimens were analyzed on two different assay platforms totaling 11 biomarkers. Logistic Support was also provided to both intramural and extramural collaborators.
- Mcl-1 inhibitor development: Developed two novel heterodimer assays that provide sensitive and precise information of mechanism of action of Mcl-1 inhibition along with 14 biomarker multiplex apoptosis panel developed earlier. Using these assays, PADIS determined the sensitivity of three cell lines, towards two lead compounds. These analyses identified the optimal in vivo model for IND enabling PK/PD and efficacy studies.
- P97: Develop Allosteric inhibitors of AAA ATPase p97 as 2nd generation drugs. PADIS developed two separate pharmacodynamics/mechanistic biomarkers to support ATPase inhibitor development.
- CHOP Sandwich Immunoassay: C/EBP homologous protein (CHOP), also known as growth arrest and DNA damage-inducible gene 153 (GADD153) is an unfolded protein response (UPR) stress marker and has been shown to transcriptionally upregulated in nucleus upon proteasome blockage. Developed a sandwich assay on Luminex platform using an N-terminal monoclonal antibody as capture and a C-terminal monoclonal antibody as reporter. The assays detected a 2-3 fold higher levels of nuclear after treatment of the lead compound.
- K-48 Linkage Specific Polyubiquitin Assay (Luminex): A lysine-48 linkage specific polyubiquitin is universal signal for delivery of substrate to proteasomes for degradation. PADIS developed a prototype sandwich assay using K-48 linkage specific monoclonal antibody as capture, an anti-ubiquitin antibody as reporter, and recombinant penta-ubiquitin as a polyubiquitin calibrator. Accumulation of K48 ubiquitin in tumor lysates provides a more proximal marker of proteasome blockage and optimal PD biomarker.
- Demonstrated utility of multiplexed apoptosis panel assays to measure UPR/ER stress induced apoptosis.
- Drug Combination Studies BTB/DCTD: To study cell death mechanisms in NCI ALAMAC drug combination studies, PADIS utilized multiplex apoptosis panel representing the intrinsic apoptosis pathway to first survey, and subsequently validate, PD biomarkers that correlate with efficacy. Multiplex analysis of paclitaxel and nilotinib combination, which has demonstrated unexpected, but robust additive/synergistic efficacy in breast cancer models, showed a lack of activation of intrinsic pathway and a caspase-3 independent cell death. Preliminary results suggest an alternative mechanism of cell death caused by combination.
- New monoclonal antibodies targeting key pharmacodynamic biomarkers, which cannot be measured currently, has become a standing activity for PADIS.
  - Monoclonal antibody molecular cloning and research level production (30 mg) was completed for pT1989ATR Clone LBR1011 7A7; there are now 3 production lots on hand.
  - Work continued on demonstration of Fitness for Purpose of the EMT/SC MAbs generated to a set of transcription factors associated with EMT and drug resistance in solid tissue cancers.
  - EMT/CSC antibodies were applied to xenograft tissues derived from the non-small cell lung cancer (NSCLC) tumor line, NCI-H596, implanted in hHGFscid/scid, hHGFki/scid or hHGFki/ki mice to examine HGF-induced changes in EMT factors, CSC markers, as well as pY1235-MET expression in vivo.

- Detected varying levels of EMT/CSC marker expression, including CD44, CD133, ALDH, and GSC, in the membrane or cytoplasm of noninvasive regions of H596 tumors in hHGFscid/scid mice.

CTC analysis and separation/device development

- Completed Validation of the Apostream / Nikon / Definiens methodology for capture and identification of Circulating Tumor Cells (CTCs) and validation against tumor biopsies from the same patient population. Completed work using two orthogonal approaches: demonstration of concordant results on a 5 channel CellSearch system (using MAb capture vs the Size/Charge separation principal used by the Apostream) and concordance with biopsy results for the EMT assay as reported above. Two manuscripts are under development.
- Completed development of a 12 channel capable method for CTC analysis, employing the ImageStream (Amnis) imaging flow cytometer in tandem with the Apostream platform. This allows rapid quantitative and cell image analysis of greater than 90 percent of the cells obtained from a typical 4 mL patient specimen. Analysis includes bright field phase contrast, fluorescence phenotypic markers, validated PD markers, and the EMT panel (ECAD, Vimentin, Cytokeratin). Topotecan or Gemcitabine-treated cancer cell lines were spiked into PBMC fractions of whole blood, which were then purified on Apostream and analyzed on the Amnis for this validation. In the process, evidence for generation of the transitional state of EMT was demonstrated in Gemcitabine treated cells.
- A subcontract was issued to Apocell, Inc., for development of a stable, fluorescent calibrator for monitoring Apostream performance (yield and purity) on a daily basis. The same microbeads can also be used to check imaging camera calibration. Two pilot lots of the bead calibrators were generated, and the first calibrator production run is anticipated in September.
- An Apostream® system was transferred to the specimen processing laboratory in Building 10 on the NIH Bethesda campus for support of ongoing DTC trials, and operation of the device was validated.

Assay community training and transfer

PADIS continued its support of the training mission and completed the first training class on the tissue extraction and subcellular fractionation methods use to prepare specimens for the Apoptosis multiplex. The MET extraction-based SOPs were posted to the DCTD website to support community transfer.

National Clinical Target Validation Laboratory

The National Clinical Target Validation Laboratory (NCTVL) serves as a clinical pharmacodynamic biomarker validation and testing center for DCTD, NCI, and is a key component for the NCI clinical biomarker program. NCTVL works closely with PADIS, NCI clinicians, investigators, and staff for laboratory correlative studies. These collaborations focus on pharmacodynamic assay development, assay transfer, assay validation, and testing of clinical specimens treated with novel small-molecular agents targeting signal transduction and DNA repair pathways. The primary focus of the lab is to provide pharmacodynamic assay support to DCTD-sponsored clinical trials in the Early Clinical Trials Development Program at NIH as well as in the Experimental Therapeutics Clinical Trials Network (ET-CTN) and to report assay results back to the trial sites.

SIGNIFICANT ACHIEVEMENTS

NCTVL supported pharmacodynamic (PD) correlative studies by testing clinical specimens with the validated PAR immunoassay (IA) and γH2AX immunofluorescent assay (IFA). PAR-IA quantifies PARP inhibition while γH2AX IFA is used as a readout for DNA breaks. A validated PAR-IA is a 96 well plate–based homogeneous immuno-antibody capture sandwich assay for PAR quantitation. γH2AX IFA is a microscope slide–based immunofluorescent assay for quantifying DNA damages in tumor biopsies, tumor aspirates, and PBMCs. Other fully transferred and validated immunoassays available in NCTVL include Top1, HIF1-a, Intact-Met, pY1356-Met, and pY1234/1235-Met. NCTVL supported several early-phase clinical trials as noted in the following table.

During FY2016, the primary clinical support efforts were for Phase 1 trials for inhibitors of PARP-1 and Topisomerase-1 (Top1). PD support for Topisomerase-1 inhibitor studies included LMP-776, LMP-400, and LMP-744 from the CTEP #8273 human trial and the COTC007b dog trial. Total Top1 levels of PBMCs and tumors were analyzed and showed reduction of total Top1 level after treatment. γH2AX induction in tumor aspirates and biopsies was also quantified. Due to the exceptional clinical response in dog lymphoma patients treated with LMP-744, the mechanism of action of LMP-744 was further studied. Collaborating with NCI scientists from the CCR nanoprotein analysis core, NCTVL is screening additional biomarkers involved in apoptosis and other pathways using an automated simple western system from ProteinSimple. Since there is no strong correlation between total Top1 reduction and tumor shrinkage, a feasibility test for Top1-DNA covalent complex (Top1cc) was initiated using a newly available antibody against DNA-bound Top1. Working with NCI investigators and PADIS staff, and using dog osteosarcoma MC and SK tumor models treated with LMP-744, we showed that quantitative Top1cc IFA is feasible in dog tumor cells. A quantitative immunoassay for identifying phosphorylation

status of Y142 of H2AX, in addition to S139, is under development. Since pY142-H2AX initiates the apoptosis pathway, the goal is to quantify pY142-H2AX levels in clinically responsive vs. non-responsive tumors.

NCTVL worked closely with PADIS scientists in the manufacturing and quality control of critical reagents, SOP revisions, hands-on training, field troubleshooting and technical assistance to support immunoassay transfer to the internal and external research community. In support of the Luminex-based multiplex immunoassay transfer and training, drug treated xenograft tumors were used for formulating and manufacturing a proficiency testing kit to monitor assay performance. The multiplex immunoassay consists of 13 biomarkers grouped into three panels targeting an apoptosis pathway. Panel 1 contains Bak, Bax, total lamin-B (intact and 45 kDa fragment), and Smac; Panel 2 contains Bad, Bax–Bcl-2 heterodimer, Bcl-xL, Bim, and Mcl1; and Panel 3 contains active (cleaved) caspase-3, Bcl-xL–Bak heterodimer, Mcl1–Bak heterodimer, and survivin. The proficiency testing kit was formulated primarily from drug-treated xenograft tumor extracts and is used in assay transfer, validation, and lab operator training. A sufficient amount of xenograft tumor extracts have been made to support manufacturing proficiency kits to run 180 assay plates. The first use of this proficiency kit will be to compare operator performance between PADIS and NCTVL. Lab staff also assists with community transfer and serves as hands-on instructors at training classes.

NCTVL worked closely with PADIS staff and initiated IFA transfer activities for the following assays: γH2AX IFA Definiens analysis, DDR2 IFA for multiple DNA repair biomarkers (H2AX, pNBS, pATR, and RAD51), pHH3, EMT, and pY15 CDK IFA. To optimize assay protocols for clinical use and to streamline assay transfer process, NCTVL technical staff have been embedded at the Frederick site and work side-by-side with PADIS staff. With newly validated Definiens software for widefield image analysis, NCTVL is now able to restrict biomarker analysis within tumor cells and improve data quality. IFA transfer/validation was completed for H2AX Definiens, pY15-CDK, pHH3 and pNBS-1 with widefield images. Installation of a new confocal microscope (Nikon Eclipse NiE) to match the microscope used by PADIS is in progress. The NCTVL multiplex IFA operation is relocating to Frederick (Building 432). This will shorten physical distance of assay transfer within the clinical PD program. Completion of facility modifications, equipment installation, and hardware and software validation is expected by the end of FY2016.

NCTVL added a project management support function under NCI's direction. Project management support focused on: 1) creating and maintaining SharePoint project sites for internal and external activities; 2) managing teams/meetings for PD, Drug Combination, Orphan Drug, Drug Chemistry, NeXT Drug, Biopsy, DCTD SBIR Topics, and External Contracts; 3) writing summaries, capturing actions and follow-ups, and 4) provided logistical support for DCTD's tumor biopsy quality initiative. The project manager also tested a new slide library system to include TRP, DTC-Clinical, and PDM, in addition to Clinical PD programs. Currently, these slides and documents from 2007 to the present are being uploaded.

Table I. NCTVL-Supported Early-Phase Clinical Trials and Project Status

Clinical Trial ID	Clinical Center	Investigational Agent	Specimen Type	Lab Status
CTEP #7286	DTC	AZD2281+Cisplatin+Gemcitabine	PAR IA, ɣH2AX IFA	Completed
CTEP #7675	DTC	ABT888	PAR IA	Completed
CTEP #7859	DTC	Batracylin	ɣH2AX IFA	Completed
CTEP #7967	Emory, UPCI, UCD	ABT888+Carboplatin+Paclitaxel	PAR IA	Completed
CTEP #7968	JHU	ABT888+Topotecan+Carboplatin	PAR IA, ɣH2AX IFA	Completed
CTEP #7977	KCI, Harvard, UMB	ABT888+ Irinotecan	PAR IA, ɣH2AX IFA	Completed
CTEP #7981	DTC	ABT888+Topotecan	PAR IA, ɣH2AX IFA	Completed
CTEP #8275	DTC	ABT888+Cyclophosphamide	PAR IA, ɣH2AX IFA	Completed
CTEP #8282	CINJ, UCD, UPCI	ABT888	PAR IA, ɣH2AX IFA	Completed
CTEP #8329	Mayo Clinic	ABT888+ Topotecan	PAR IA, ɣH2AX IFA	Completed
CTEP #8437	DTC	ABT888 + Cyclophosphamide PII	ɣH2AX IFA	Completed
CTEP #8456	UM/JHU	ABT888 + Temozolomide	PAR IA	Completed
CTEP #8472	OSU	ABT888 + Mitomycin C	PAR IA, ɣH2AX IFA	Completed
CTEP #8609	OSU	ABT888 + Carboplatin	PAR IA	Completed
CTEP #8610	DTC	EZN-2208 (PEGylated SN-38) + Bevacizumab	HIF1-α IA, Top1 IA	Completed
CTEP #8620	UPCI	ABT888 + Carboplatin + Paclitaxel	ɣH2AX IFA	Completed
CTEP #8788	DTC	ABT888 + Cyclophosphamide	PAR IA, ɣH2AX IFA	Completed

Table I. NCTVL-Supported Early-Phase Clinical Trials and Project Status

Clinical Trial ID	Clinical Center	Investigational Agent	Specimen Type	Lab Status
CTEP #8880	DTC	Pazopanib + ARQ 197	Total-Met IA, pMet IA, Dual pMet IA	Completed
CTEP #9350	DTC	MK1775	ɣH2AX IFA	Completed
CTEP #7998	CINJ	ABT888 + Cyclophosphamide	PAR IA, ɣH2AX IFA	Data Analysis
CTEP #9510	DTC	BMN673	PAR IA, ɣH2AX IFA	Data Analysis
COTC007b	Purdue/COTC	LMP744, LMP776, LMP400	PAR IA, ɣH2AX IFA	Data Analysis
CTEP #8273	DTC	LMP776, LMP400	Top1 IA, ɣH2AX IFA	On Schedule
SARC025	UCL	Niraparib (MK4827)	PAR IA	On Schedule

Pharmacokinetic/Pharmacodynamic Specimen Processing Lab

The Phase I/II Pharmacokinetic/Pharmacodynamic Support Lab handles biospecimen processing for the Early Clinical Trials Development Program and is responsible for supplying uniform blood and other biospecimen processing core support as identified in the clinical trial protocol. This includes processing for pharmacokinetic/pharmacodynamic components of clinical trials sponsored by CTEP. The group is located in the NIH Clinical Center, Bethesda, MD.

SIGNIFICANT ACHIEVEMENTS

- The lab processed over 1,350 incoming biospecimens from 136 patients and outputted over 3,300 prepared samples to various labs. The lab also maintained work coverage throughout odd hours and weekends on a routine basis for time-sensitive samples.
- The lab worked on 17 active trials and launched three new protocols in 2014/2015, including MATCH, VX970, and bortezomib/clofarabine. The lab closed out two protocols including working with various groups to share the data on hand.
- The lab took the first steps toward establishing an electronic biospecimen tracking system and received permissions to LabMatrix and have inputted TRC102 protocol data to test the system.
- The lab collaborated with PADIS to install and operate an Apostream device and currently has the machine up and running. Numerous runs have been completed to validate the machine. We will be able to process CTCs by the end of 2016.

NExT Program Support: Chemical Biology Consortium

The NCI Experimental Therapeutics (NExT) Program is a cancer drug discovery and development pipeline established by the NCI Division of Cancer Treatment and Diagnosis (DCTD) in conjunction with the NCI Center for Cancer Research (CCR). Its vision is to bring promising drugs to patients more rapidly by streamlining NCI's anticancer drug discovery and development resources under a unified governance structure (http://next.cancer.gov). The Chemical Biology Consortium (CBC) was originally assembled in 2008 to provide the drug discovery expertise, facilities, and personnel needed to deliver drug candidates for cancers or cancer targets currently not being addressed by the pharmaceutical industry. Novel, potential therapeutic agents emerging from these efforts may be selected for preclinical and clinical development within NExT. The CBC member institutions participate in the Consortium and on project teams through Task Order agreements administered as subcontracts by Leidos Biomedical Research, Inc. (Leidos Biomed).

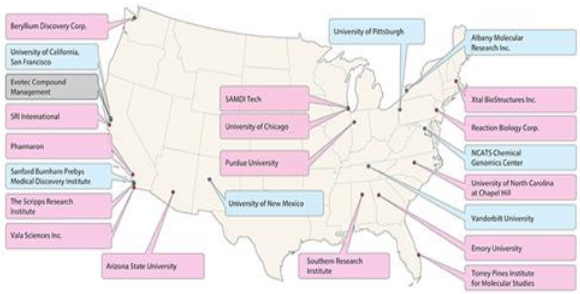
The CBC Scientific Project Manager (SPM) Group works closely with the NCI to ensure that Discovery Projects are established, supported with the appropriate scientific staff and expertise, and are completed within the established budget. Through an RFP and competitive selection process, the best-suited CBC Centers are identified for participation on a project team. Once established, the SPM then coordinates activities and facilitates communication among project team members to ensure the biology and chemistry tasks that are essential for the project's progress are carried out. The SPM also monitors and evaluates the subcontractors' technical progress and assists with resolution of technical problems.

In 2015, the NExT CBC program received a favorable review, and DCTD decided to renew the program. An open competition was conducted to identify institutions that could provide the scientific and technical expertise needed to advance novel therapeutic targets, or hit compounds from the early stages of drug discovery, to the point of selecting a clinical candidate. NCI could then take any qualified candidate through the preclinical development studies required for IND filing.

The decision to renew the CBC launched a major, nine-month-long procurement activity. The process to select this integrated network of centers from academia, nonprofit, and commercial organizations began in July 2015. An RFP was developed/issued, and proposals were received. A Source Evaluation Group (SEG) of scientists from NCI, Leidos Biomed, and outside institutions was assembled to evaluate the proposals. Six of the bidders

were selected as Dedicated Centers (awarded Master Service Agreements) and 15 institutions were selected as Specialized Centers (awarded Basic Ordering Agreements). All agreements were established by the April 1 target date.

The renewed CBC has almost twice as many member institutions as the original program and has expanded its expertise substantially in the areas of protein structural biology, medicinal chemistry, and animal pharmacology. The illustration below (from http://next.cancer.gov/ shows the Specialized Centers (pink) and the Dedicated Centers (light blue), which includes the governmental Dedicated Center, NCATS.



NExT Program Support Reorganization: As part of the NExT CBC renewal, additional project management responsibilities for CBC discovery projects were assimilated into the SPM group, and a Director of CBC Support Programs was hired to serve as the single point of contact with NCI for program activities. The SPM group is now more directly involved in helping NCI organize and plan CBC Steering Committee Meetings and meetings of the Special Emphasis Panel (SEP). The group is also now more closely linked with the accounting and finance groups, which will better ensure the accurate tracking of budgets and expenses on CBC projects.

NExT CBC Projects: The SPM Group provided scientific and technical project management support for 10 NExT projects during the year (see the following table). The work on these projects is being carried out at locations across the consortium, with newly affiliated Centers being integrated to provide new and different capabilities to benefit the projects during the third and fourth quarters of FY2016.

	Project Name	Participating Institutions	Project Stage	Status
1	AAA ATPase p97	UCSF, UPCDC, UCLA, AMRI	SDS & LI	Active
2	Artemis Endonuclease	SBP, SR, SRII, UPCDC, AMRI	SDS	Active
3	ATG4B	SBP, UCSF, UPCDC	ESD	Close out
4	GBM-PPI	Emory, UCSF	TAF	Active
5	IDH1	NCATS, UNC	SDS & LI	Active
6	KDM5A/B	Emory, NCATS	SDS	Active
7	LDHA	NCATS, SR, VCDC, Beryllium, UNM, UAB	SDS & LI	Active
8	Mcl-1	VCDC, NCATS	SDS & LI	Active
9	Taspase1	UCSF, VCDC, SRII, Beryllium, TPIMS	FB-SDS	Active
10	WDR5-MLL1	VCDC, NCATS, AMRI	SDS & LI	Active

Participating Institutions: Southern Research Institute (SR); Sanford Burnham Prebys Medical Discovery Institute (SBP); SRI International (SRII); University of Pittsburgh Chemical Diversity Center (UPCDC); Emory University (Emory); University of California San Francisco (UCSF); National Center for Advancing Translational Sciences (NCATS); University of North Carolina (UNC); Vanderbilt Chemical Diversity Center (VCDC); Torrey Pines Institute for Molecular Studies (TPIMS); University of New Mexico, Health Sciences Center (UNM); University of Alabama, Birmingham (UAB).

CBC Compound Registration and Project Data Capture

The SPM Group provides technical support to the NExT Program initiative on CBC compound submission and structure registration. Improvements continue to be made in this system in terms of speed and convenience, which in turn has led to increased timely submission of compounds to the NCI-Chemotherapeutics Agents Repository by the CBC Centers. Registration of structures and collection of samples of high value compounds are important deliverables for chemistry-based subcontracts. In accordance with NCI direction, the SPMs have led efforts to capture project data from the CBC Centers in an NCI database. Several improvements were introduced last year that enabled different types of data and results to be collected and then analyzed. For example, in vitro ADME data and pharmacokinetic data can now be uploaded through a simplified template. In conjunction with this data capture and storage effort, the SPMs continue to

refine and improve data-surfacing capabilities by using and receiving training on D360, a powerful data retrieval and analysis tool that is made available to the NCI staff and all CBC Centers. The availability of a centralized database housing project team data is particularly valuable for multicentered project teams, fostering collaborative discussion among the participating centers on the teams.

Project Management Office

The Project Management Office played a role in supporting the drug discovery and development efforts at NCI by integrating standardized, business-focused project and portfolio management practices and tools into existing drug discovery and development programs. One of these programs is the NExT Program, which consists of drug discovery projects from the Functional Biology and Chemical Biology Consortia. As a part of a reorganization effort directed by NCI, the Project Management Office was disbanded in the third quarter of FY2016.

Medical Writing Unit

The Medical Writing Unit (MWU) provides scientific writing and clinical protocol support to DCTD. Its primary functions are to write and submit clinical protocols and informed consent forms for Cancer Therapy Evaluation Program (CTEP) and NCI IRB review and approval, and to write and publish preclinical and clinical manuscripts arising from DCTD research activities. In addition, MWU prepares documents (abstracts, meeting summaries, PowerPoint presentations, pamphlets, posters, and notification letters) for the DCTD Developmental Therapeutics Clinic (DTC), the DCTD Pharmacodynamics Research Group, and the NExT Program. Specific activities are summarized below.

- MWU currently supports 31 Phase I/II clinical protocols for DTC. In the past year, MWU efforts resulted in four new protocol approvals. The Molecular Profiling–based Assignment of Cancer Therapy (MPACT) clinical trial was also opened as a multicenter trial within the Experimental Therapeutics Clinical Trials Network. Four new protocols, including two immunotherapy studies, are undergoing regulatory review.
- MWU provided scientific writing, data analysis, and editorial support for eight published manuscripts and an entire issue (10 individual chapters) of Semin Oncol written by DCTD and Leidos Biomed staff and devoted to various aspects of pharmacodynamics in clinical cancer research.
- The MWU substantially revised clinical protocol procedures and eligibility criteria language as part of a joint initiative with PADIS and the DTC to improve the quality and quantity of biopsy tissue collected for research. This effort supports "longitudinal" research biopsy collection and use such that research tissue collected at disease progression or from other sources can (if of sufficient quality) serve as a baseline sample for subsequent DTC trials, potentially obviating the need for a patient to have another biopsy procedure.

Cancer Diagnosis Program

Support Provided by the Clinical Research Directorate

Biospecimen Research Group

Genotype-Tissue Expression (GTEx) Scale-up Phase II

The National Institutes of Health (NIH) Common Fund's Genotype-Tissue Expression (GTEx) project aims to study human gene expression and regulation in multiple tissues and is providing valuable insights into the mechanisms of gene regulation and, in the future, its disease-related perturbations. Leidos Biomed completed its role in procuring 960 cases for GTEx, and now continues to provide support by developing and implementing the GTEx Legacy plan.

An open competition was conducted, and the Broad Institute will take on the responsibility of marketing, distributing, and housing the GTEx collection. The institute also committed to supporting the collection beyond the end of the Leidos Biomed subcontract period of performance. This year, the Leidos Biomed GTEx team also developed a light, open-source version of the Comprehensive Data Resource, along with extensive documentation. The GTEx vocabulary is now established in NCI's Cancer Data Standards Registry and Repository (caDSR) and is being published to the NIH BioPortal, Obo Foundry (OBIB), and National Center for Biomedical Ontology (NCBO) BioPortal.

In addition, the Ethical, Legal, and Social Implication (ELSI) Study staff continues to work on publications related to its exploration of family decision-maker comprehension of bio-banking research and areas where consent-administrator training impacts recollection and consent rates. Another key part of the GTEx ELSI sub-study is community engagement, and the project includes input from two community advisory boards, one of which focuses on issues affecting the Hispanic community.

Biospecimen Pre-Analytical Variables Program

The Biospecimen Pre-Analytical Variables (BPV) Program entered its final year with a concerted focus on the analysis of the BPV specimens. The well-annotated kidney, colon, ovarian, and lung samples created a sample bank with rich metadata. The sample bank was used for downstream analysis that ultimately uncovered the effects of pre-analytical variables on different analytes using a variety of analysis platforms, such as protein-based analysis with mass spectrometry; RNA analysis via transcriptome sequencing and custom TaqMan OpenArrays; plasma-based research using Luminex and mass spectrometry; and metabolomics profiling. New

contracts were executed, implemented, and closed in record time to facilitate the previously mentioned analysis studies. This research effort has yielded surprising findings that should be an important contribution to the field. In addition, a summary analysis report was prepared and submitted to NCI.

Developmental Therapeutics Program

Support Provided by the Biopharmaceutical Development Program

Process Analytics

Process Analytics (PA) functions both as a method development laboratory that interacts directly with project scientists and customers during the feasibility and development phases of a project and as a current Good Manufacturing Practices (cGMP)–compliant analytical laboratory for facility environmental monitoring, raw materials testing, in-process testing, nonclinical, and clinical product quality control (QC) testing. During this reporting period, PA processed approximately 8,750 samples from approximately 1,460 unique test requests. Clinical cGMP drug product lots from the Tetanus-CMV peptide, ganitumab, PVSRIPO MVB, TA-CIN, and TA-CIN/GPI-0100 projects were analyzed by PA during the reporting period. In addition, PA conducted analytical development and research lot testing for the RLIP76, mrhIL-7, ch11-1F4, and EBV gp350-Ferritin projects. The PA group also continued to support serious adverse event (SAE) and product excursion testing for the Panitumumab-IRDye, rhIL-15, and HuMikβ1 clinical studies, as well as conducting out of specification (OOS) manufacturing investigations for the Tetanus-CMV gelling and TA-CIN pH and microbial contamination issues. During the reporting period, PA conducted studies to re-certify cell banks and bulk drug substances from external manufacturers (TA-CIN) and manufactured in-house (EBV gp350-Ferritin, mrhIL-7, and RLIP76). PA also finalized a report for U.S. Food and Drug Administration (FDA) review detailing the deep sequencing analysis of seven PVSRIPO product lots, including a peer-reviewed publication effort lead by Duke University, and continued to provide supplemental support to United Therapeutics Corporation (UTC) for licensed monoclonal antibody ch14.18. PA also prepared clinical administration compatibility studies for rhIL-15 and conducted accelerated stability studies for the Tetanus-CMV peptide and RLIP76 projects. In addition to these efforts, the PA laboratory staff supported the ongoing analysis of 32 clinical product lots in the Biopharmaceutical Development Program (BDP) Stability Program. Significant efforts were made during the reporting period to reduce the Stability Program backlog, which in a small number of cases exceeded a one-year delay from the time of submission. These efforts included detailed proposals for additional PA staff and increased analytical out-sourcing, both of which require Biological Resources Branch (BRB)/Division of Cancer Treatment and Diagnosis (DCTD) approvals for implementation. Other immediate mitigation efforts included a temporary re-assignment of BDP Development staff to PA, utilization of BRB resources for generation of study reports, additional program harmonization, and elimination of nearly a dozen nonclinical lots from the Stability Program. The internal mitigation efforts resulted in a significant reduction in the stability backlog by the end of the reporting period and these efforts will be continued into FY2017.

PA staff members also continued to act as project scientists for several new and legacy projects. The mrhIL-7, PVSRIPO, ganitumab, Tetanus-CMV peptide, RLIP76, TA-CIN, and EBV gp350-Ferritin projects have been the major focus of PA cGMP analytical efforts during this reporting period. In addition to testing and releasing five cGMP product lots during the reporting period, PA scientists developed several new analytical methods for pre-clinical development of EBV gp350-Ferritin, including: SEC-HPLC, SEC-MALS, DLS, Western blotting, gel analysis, peptide mapping MS/MS, activity bioassays, and a variety of other product characterization tests. The activity assay efforts with the RLIP76 project also continued in the reporting period resulting in a completed Antioxidant Response Element (ARE) pathway protocol for drug substance release testing, as well as the development of a murine cell line variant of the assay to support nonclinical toxicology studies. In order to support the transfer of the PVSRIPO viral manufacturing process to a third-party contract manufacturing organization (CMO) (via Astari/Duke Univ.), PA staff with assistance from BDP Development and the NCI Technology Transfer Office wrote and filed two U.S. patent applications for final United States Patent and Trademark Office (USPTO) review in 2016. PA also conducted early development and review activities for the proposed NCI (ch11-1F4, LMB-100), NCATS (four new projects), NIAID (EBV-Ferritin ‘phase 2’) as well as other prospective projects in FY2017.

Product testing and certification during the reporting period.

PA approved 12 master specifications, representing eight projects, and 13 new or revised certificates of analysis (COAs), including the following:

New COAs for:
Tetanus-CMV Peptide
Ganitumab
PVSRIPO MVB and Ref. Std.
PVSRIPO HSA Diluent
TA-CIN and Placebo
TA-CIN/GPI-0100
EBV gp350-Ferritin MCB
mrhIL-7 MCB
RLIP76 MCB

Operations

Because of the limited amount of labor resources available and in order to maximize those resources, BDP operations comprised a pool of personnel available to perform a variety of operational tasks. Since the demands on different elements of technical operations (manufacturing, testing, and research and development) vary widely at any given time, the technical staff maintains broad expertise among the employees so that the expertise can be effectively utilized in a variety of capacities to meet the current demands of the NCI workload. The validation, environmental monitoring, water monitoring, and raw material testing duties are all performed by this labor pool. The same labor pool performs production operations, technology transfer, and scale-up, with specific personnel assigned duties based on individual skill sets. Additionally, this group shoulders the burden for a variety of other tasks that other groups lack sufficient resources to perform, such as periodic review of equipment logbooks, assisting with quality assurance (QA) data input and tracking, etc. Finally, this group also provides expertise in streamlining and automating operations across the organizations so that all groups may perform their tasks as efficiently as possible. Some of the noteworthy accomplishments of the past year include:

- Improvements in the system used by QA for management of standard operating procedures (SOPs), including a significant amount of process automation.
- Implementation of a new Engineering Event (EE) system for QA that allows for more efficient and robust processing of these events.
- Initiation of a retrofit to the control system on the 1000 L bioreactor, which is nearing completion.
- A good manufacturing practice (GMP) fill of over 9,000 vials of Ganitumab product for clinical use.
- Assistance in the development work scopes for the PTEN Long, EBV-Ferritin, and RLIP 76 projects.
- Production of GMP grade master cell banks to support the EBV-Ferritin and RLIP 76 projects.
- Production of GMP grade plasmid to support the EBV-Ferritin project.
- A GMP fill of more than 270 vials of TA-CIN product and 680 vials of placebo for clinical use.
- A GMP fill of more than 400 vials of GPI100 product for clinical use.
- Production of a 150 L scale fermentation and subsequent purification of RLIP 76 product for R&D use.

Development

RLIP76, a radiation exposure amelioration project from the National Center for Advancing Translational Sciences (NCATS), the BDP has completed Milestone 1. Approximately 1.5 gm of drug substance was shipped to AMRI to be lyophilized into a drug product. This will subsequently be tested in a rodent efficiency model. Approval was obtained for the 15 gm (drug substance) scale up run. A 110 L fermentor harvest was completed to be followed by downstream purification.

Endotoxin free recombinant PTEN-Long protein was prepared using E. coli harvests and tested in cellular assays. The project did not proceed further to scale up and clinical trials.

NIAID's project for EBV (gp350)-Ferritin vaccine completed Milestone 1. 10 mg of drug product was provided to the PI for animal studies. This is currently in progress and a demonstrated vaccine efficacy in an animal model would trigger Milestone 2—scale up of the process.

Biopharmaceutical Quality Assurance and Regulatory Affairs

BDP maintains a quality system that is compliant with FDA requirements for manufacturing Phase I, II, and nonpivotal Phase III investigational products. QA responsibilities are distributed between the QA staff and the administrative and technical operations staff. We continue to evaluate our quality systems and investigate ways to improve efficiency while maintaining quality with significantly reduced resources.

The BDP is fully operational at the Advanced Technology Research Facility (ATRF). Because of resource limitations, the eight manufacturing suites are maintained but only those in use are monitored. It takes two to six weeks to ready a suite for GMP manufacturing.

Multiple products manufactured by the BDP are now in late-stage clinical trials and/or undergoing commercial licensure activities. The FDA and European Medicines Agency (EMA) approved the commercial licensing of ch14.18 (now known as dinutuximab, generic name, and Unituxin, brand name) for UTC. Eleven lots of ch14.18 were manufactured at the BDP for preclinical and clinical studies. Both the BDP-supplied product and the assistance that BRB and BDP personnel provided to UTC were significant factors that led to FDA approval.

Quality Assurance Auditing

Quality Assurance (QA) Auditing releases product for clinical and toxicological use, and provides compliance oversight of BDP cGMP production, testing, and support operations (including conducting internal audits to ensure compliance with cGMP), as well as external contract manufacturing and testing facilities.

QA Auditing approved product release documentation for three final vialed products for human clinical trials, one master viral bank, one working cell bank, and two master cell banks for cGMP manufacturing use. To accomplish this, QA reviewed 20 master production records, 44 batch production records, 18 master specifications, and 11 COAs, and conducted 10 production area releases and in-process audits of critical manufacturing operations.

QA processed approximately 2,000 **documents** to support various BDP cGMP, good laboratory practice (GLP), and laboratory activities. QA continues to process and track more than 25 types of documents. Improvements in database systems continue to be evaluated to increase efficiency.

Products Manufactured and Released by BDP

Product	Type	Number Released
TET-CMV Peptide	Final Vialed Product	618 vials
PVSRIPO	Master Virus Bank	53 bottles
Ganitumab	Final Vialed Product	8,719 vials
EBV gp350 Ferritin	Master Cell Bank	199 vials
TA-CIN Placebo	Final Vialed Product	567 vials
E. coli HMS174 (DE3)	Working Cell Bank	500 vials
E. coli DH5a IL-7 Plasmid	Master Cell Bank	205 cryovials
TOTAL	7 Lots	10,861 vials

Quality Engineering and Validation

Quality Engineering and Validation (QE&V) oversight of facilities, utilities, equipment, and related supporting activities is performed by our permanent quality engineer and a one-year contract quality engineer who started in May 2016. Responsibilities include managing the equipment calibration program, validation program, engineering controls, and failures to ensure that facilities, utilities, equipment, and supporting activities are suitable for use. Other BDP staff assist with the calibration data review, environmental excursions, and authorship and execution of validation protocols as needed to minimize schedule impact from the huge workload. Priority is given to those pieces of equipment and processes related to upcoming and critical operations. Requalification of systems is performed by the BDP. Utilizing shared resources and scheduling activities for manufacturing and validation takes careful coordination.

QE&V has been instrumental in maintaining ATRF operability and validation. This requires coordination of BDP technical labor resources and FME staff for calibration, preventative maintenance, and repair. Qualification and validation for utilities and all classes of equipment have been executed. Routine monitoring of the environment and utilities has demonstrated that the facility and utilities operate in full conformance with FDA requirements.

Benefits from the new quality engineer are already evident as work has resumed on Annual Certifications, calibration review is staying up to date, and the efforts are allowing added focus to be placed on addressing audit findings and closing of quality gaps.

Regulatory Affairs

Regulatory Affairs (RA) provides regulatory agency submission documentation to describe the manufacture and testing of BDP products to meet regulatory requirements. Submission strategies and regulatory guidance are provided to expedite regulatory agency review and move products into clinical trials.

Regulatory support was provided for six BDP products. Key documents were prepared for submission to regulatory agencies, including Chemistry, Manufacturing, and Controls (CMCs), Clinical Trial Application (CTA) CMCs (Health Canada), responses to regulatory agency questions, and related documents. Additional support for the tracking of stability data and annual reports was provided for 28 products.

Major achievements this year have included regulatory support for the Ganitumab, HSV C134, TA-CIN (HPV16 L2E7E6 fusion protein), Tetanus-CMV, and the PVSRIPO projects. Investigational New Drug (IND) applications CMC amendments were completed for PVSRIPO (treatment for glioblastoma), Tetanus-CMV (a fusion peptide vaccine for the prevention of infectious cytomegalovirus), and Ganitumab (for the treatment of patients with Ewing sarcoma). A full IND CMC was completed for HSV C134 (treatment for malignant glioma and other tumor types). CMC support and technology transfer continue for the PVSRIPO treatment for glioblastoma, which received FDA's Breakthrough Therapy Designation in May 2016.

RA continues to maintain a current facilities drug master file (DMF) that is on file with the FDA for the ATRF facilities. This document describes the facilities, utilities, and critical process flows used in the manufacture of BDP products.

Support Provided by the Applied and Developmental Research Directorate

Natural Products Laboratory

The Natural Products Support Group provides scientific and technical support to the Developmental Therapeutics Program (DTP), DCTD, NCI. The Extract Production Lab carries out all grinding, extraction, drying, and freeze-drying of plant, marine, and fungal biota specimens. The Drug Processing Lab weighs pure chemicals and extracts, produces both 96-well and 384-well microtiter plates of extracts and pure drugs for anticancer screenings, ships and receives extracts, drugs, and plates, develops solubilization methods, and processes and delivers extracts and drugs for animal testing. The Fungal Metabolites Lab performs fermentations for screening, assures culture purity, performs cryo-preservation of fungal stocks, optimizes titration of bioactive metabolites, and carries out scale-up fermentations for drug production. The Natural Product Chemistry Laboratory provides analytical support; purifies compounds of interest for testing; identifies new natural products; makes chemical modifications of

compounds with biological activity; and performs scale-up isolations of compounds of interest to DTP for further biological evaluation.

In addition, during the last year, a large amount of the NPSG's time and resources have been shifted towards methods development and implementation for the generation of a new fraction library as part of the "NCI Program for Natural Products Discovery." Once completed, this 1,000,000 natural product fraction library is expected to be the largest natural product-based resource of its kind in the world available for screening.

Extract production and drug processing

During FY2016, new extract production totaled 617 (34 plant, 304 marine, and 617 fungal extracts). Plating of new extracts gave eight new plate maps, which increases the screening library to 2,500 unique plates. Resupply of existing platemaps produced more than 2,400 plates. More than 2,900 microtiter plates were produced.

In support of the NCI-60 primary anticancer screen, sample preparation was 480 total test slots (one-dose equivalents) per week. Drug preparation support to in vitro anticancer testing totaled 1,438 compounds for multidose 60-cell testing and 9,999 compounds for one-dose 60-cell testing. Specialty plate production requests (i.e., Natural Product Set IV and 384 SD-9 replicates for compound validation screen, IOA plate set, INV plate set, and cherry-picked set of the AOD and IOA for long-term exposure assays) and other special requests (i.e., nonstandard vehicle or dilution sequence compounds) have increased this year. Testing has continued at slightly more than 50 percent of capacity committed to testing natural product extracts and fractions for Biological Evaluation Committee (BEC) projects.

In support of DTP in vivo anticancer testing, there were 108 synthetic experiments (260 drugs tested in 7,207 dosing vials) and one natural product experiment (one drug tested in 18 dosing vials) prepared. Solubility studies were conducted on 38 synthetic products and one natural product.

Fungal Metabolites Laboratory

The Fungal Metabolites Laboratory (FML) provided 295 microbial extracts from 60 organisms, resulting in over 260 liters of fermentation broth. Three large-scale fermentations were completed: aurantimycin (12 L of fermentation broth), herboxidiene (12 L of fermentation broth), and spliceostatin (1 L of fermentation broth).

The laboratory conducted a comprehensive methods development study focusing on the improvement of the current extraction procedure and has implemented the new extraction methodology as the current standard operating procedure. The new methodology has resulted in a significant improvement on the quantity and quality of the microbial extracts.

In addition, the laboratory has started work on a subcontract with the University of Oklahoma as part of the Citizen Science Fungal Program. To date, over 600 fungi have been processed and determined to be pure and viable, resulting in more than 6,000 cryovial stocks for future fermentations.

Natural Products Chemistry Laboratory

The laboratory investigated 70 natural product extracts for the isolation and identification of anticancer active natural products, as requested by the BEC. The lab identified 49 pure natural products that have NCI-60 cell activity, with six of those re-submitted for in vivo testing and evaluation as potential anticancer drug candidates. The laboratory has also completed large-scale isolations of microbial natural products aurantimycin, herboxidiene, and spliceostatin, and has supplied the pure compounds to the DTP for further in vitro and in vivo biological evaluations.

The laboratory implemented new bioinformatics-guided approaches for the prioritization of lead projects from NCI-60 data. The assay-driven, bioinformatics-guided approach was validated through the use of LCMS- and NMR-based metabolomics, as well as bioassay-guided isolation work.

Pre-fractionation Project

The laboratory completed an implementation of automated pre-fractionation of natural product extracts that involved the use of existing and new equipment. Four new instruments were installed that included a Tecan Evo liquid handler, two automated tube weighing systems, and an automation friendly capper/decapper. The liquid handler was customized to perform high-throughput, automated liquid chromatography work capable of pre-fractionating 88 samples at a time. Production at a rate of 1,848 fractions per week was started in March, and more than 35,000 fractions have been produced to date.

Work on second-stage rapid purification was initiated, two new LC-MS systems were installed and the chromatographic methods development is in progress.

In Vitro Evaluation and Molecular Pharmacology

In Vitro Screening Group

The In Vitro Screening Group comprises the NCI-60 Screening Lab and the Target Validation and Screening Lab. The NCI-60 Screening Lab is responsible for running supplied samples against 60 cancer cell lines to identify valuable compounds for development as anticancer agents.

NCI-60 Screening Lab

The aim of the NCI-60 screen is to identify, for further evaluation, synthetic compounds and natural product samples showing selective growth inhibition or cell

killing in specific cell lines in the NCI-60 cell line panel. The screen can also be used to evaluate drug combinations in order to identify those that produce an additive or synergistic effect on tumor cell line growth and survival.

The NCI-60 screen consists of a three-step testing process that starts with an initial single-drug dose screen against all 60 cell lines. Drugs that show activity in the one-dose assay are retested in a five-log-dose concentration test. The results of this assay are used to determine if the drug is selected for a second confirmatory five-dose assay. Specific achievements are summarized below:

- Performed testing on 2,260 rug plates containing either two five-dose samples or 10 one-dose samples
- Performed one-dose testing on more than 3,936 new synthetic compounds and 5,616 natural products. These assays also included more than 1,078 assays of the internal drug standard that is included in each one-dose testing plate.
- Tested more than 587 synthetic compounds, 554 natural products, and 50 internal drug standards in the five-dose screen, with greater than 150 compounds submitted for the confirmatory five-dose assay.
- Provided greater than 550 lines, including making approximately 568 T150 flasks, and prepared 190 96-well plates with cells for use by other DTP support laboratories.
- Prepared over 274 samples lines that are available for testing to verify identity of cells.
- Prepared and shipped one set of 60 cell lines with one sample each of RNA to investigators approved by the DTP Molecular Targets Committee.
- Prepared and shipped 20 sets of 60 cell lines with one sample each of DNA to investigator approved by the DTP Molecular Targets Committee.
- Prepared and shipped 20 flasks of viable cells to an investigator approved by the DTP Molecular Targets Committee.
- Prepared and shipped 25 ml of conditioned media for 60 cell lines to investigators approved by the DTP Molecular Targets Committee.
- Prepared and shipped RNA extracts in Trizol solution for 60 ell lines to an investigator approved by the DTP Molecular Targets Committee.

Target Validation and Screening Group

- Developed and utilized new 3-D in vitro models to examine the activity of select agents in assays. Identified suitable end points to examine drug efficacy and demonstrated that the 3-D models could withstand prolonged drug exposures compared with 2-D models (3-D spheroids were exposed to compounds for up to 15 days).
- Completed screening of the entire NCI60 cell line panel against more than 800 compounds, each monitored at nine concentrations using an automated HTS robotic platform. Efforts are ongoing to compare observations to multiple PDX lines using longer drug exposure times in monolayer cultures (seven-day exposure) and in 3-D models (11-day drug exposure).
- A manuscript based on the data obtained from the small-cell lung cancer (SCLC) screening was submitted and published, and all of the data from this screen was uploaded to a website for access by external researchers via a separate public-facing website. External researchers will be able to use the data from the compound screen along with the genomics data available from the website to develop and test novel therapeutic hypotheses.

Molecular Pharmacology Group

The mission of the Molecular Pharmacology Group is to evaluate the molecular responses of well-annotated patient-derived model cancer cell lines (PDM lines) to specific regimens (i.e., MPACT drugs: trametinib, everolimus, a combination of temozolomide and ABT-888, and a combination of MK1775 and carboplatin) and to highlight novel drug combinations based on the activation of specific signaling pathways. In addition, the group employs cutting-edge techniques (i.e., clustered regularly interspaced short palindromic repeats [CRISPR]/Cas9) to alter the expression of specific targets and, therefore, identify the molecular pharmacology responses that determine sensitivity to specific therapeutic agents. The laboratory has the ability to support validation and modulation of genes and target proteins when deemed necessary, has extensive molecular biology experience, and expertise in the cellular microenvironment (i.e., hypoxia).

SIGNIFICANT ACHIEVEMENTS

- Evaluated a bladder PDM model that is uniquely sensitive to temozolomide (TMZ). Loss of MGMT expression in glioma, and possibly in colorectal carcinoma, is considered a predictive biomarker for response to alkylating agents, such as dacarbazine and TMZ. MGMT protein expression was not detected in the PDM model. To elucidate unique determinants of hypersensitivity to TMZ beyond MGMT expression, DNA damage responses elicited in this model were examined. TMZ activated ATR and ATM signaling pathways paralleling the pharmacodynamic response of apoptosis biomarkers. Inhibition of ATR signaling pathway, but not ATM-dependent responses, resulted in increased sensitivity to TMZ. Other models that lack MGMT expression, but are TMZ sensitive, showed a transient activation of the ATR and ATM signaling pathway that was not linked to toxicity. The group then inhibited ATR signaling and observed a sensitization of this model

to TMZ treatment. Moreover, in PDM models that are expressing MGMT, pharmacological inhibition of MGMT resulted in sensitivity to TMZ in combination with ATR inhibitors, but not to TMZ single agent. These results highlight the importance of ATR-dependent responses in TMZ sensitivity and warranted in vivo experiments to confirm molecular responses and efficacy in PDMs.

- Currently evaluating additional PDM models (as they become available) and cell lines from the NCI60 panel to better understand the molecular determinants of sensitivity to the combination of TMZ and ATR inhibitors. Verified that not only lack of MGMT expression, but also a particular MMR status is required for sensitivity to TMZ in combination with ATR inhibitors.
- RNA-guided genome engineering using the CRISPR-Cas9 system has yielded an unprecedented ability to perform site-specific editing in a variety of genomes. The group is taking advantage of a lentiviral-based, doxycycline-dependent system to introduce Cas9 in difficult-to-transfect lines and express Cas9 in an inducible fashion. Using this system, the group has created several clonal plastic PDM platforms that are amenable to editing of choice following the introduction of a specific sgRNA to the target of choice and the induction of Cas9.
- Successfully created clones with multiple copies of kinase dead ATR by introducing frameshift using CRISPR/Cas9 editing techniques. These clones have significantly reduced ATR signaling responses and showed an increased drug sensitivity. Unfortunately, full editing of ATR was not achieved possibly because ATR is required for normal cellular functions.
- CRISPR/Cas9 was used to create ATM kinase dead clones. Successfully isolated a clonal line that has one kinase dead copy (out of two) by introducing frameshift mutations. This clone has a significant reduction in ATM signaling responses and showed increased sensitivity to ATR inhibitors. Even in this case, full editing of ATM was not achieved. Moreover, heterozygous editing was particularly difficult to isolate, and ATM editing resulted in loss of proliferation, suggesting that the dominant negative effect of the introduced editing is deleterious to cells.

In Vivo Evaluation Group

The central mission of the In Vivo Evaluation Group involves developing a repository containing patient-derived tumor models (PDMs). These PDMs will encompass 75–100 cancer subtypes derived from patient biopsies/resections and circulating tumor cells (CTCs) isolated from blood samples. Ultimately, this repository will contain both tumor fragments and in vitro cell cultures (extending also to paired cancer-associated fibroblasts).

First-generation xenograft tumors from patient material are re-implanted into at least 10 new NSG mice to scale material. The goal is to bank at least 200 vials containing tumor fragments from each "xenopatient," to accommodate future demand from extramural investigators. Representative specimens are either flash frozen or fixed in 10 percent buffered formalin or OCT media to accommodate hematoxylin and eosin staining/pathology and microarray/DNA/exome analyses. Each model will (1) undergo histopathologic analysis relative to the original patient tumor; (2) be examined by PCR for the percentage of mouse tissue present; and (3) be screened for the presence of mycoplasma and mouse/human pathogens. Models that have been delinked from patient identification information will be subjected to cDNA microarray and next generation sequencing (NGS) assays, along with confirmation of patient origin using short tandem repeat analysis.

Several approaches for establishing in vitro cultures for each model are being evaluated in order to maximize viability and maintain heterogeneity. Approaches include utilizing different matrix supports, growth factors, and media with serum or serum replacements. To establish primary cell cultures, tumor fragments are subjected to collagenase digestion, or a small piece of biopsy/resection is placed directly onto the Matrigel. In addition to isolating tumor cells, the group also isolates and purifies corresponding cancer-associated fibroblasts. Flow cytometry, using antibodies against human HLA-A, B, C, mMHC class I, CD9, mCD9, CD90, EpCAM, and CD24, provides initial information regarding the level of mouse contamination and the level of human tumor or fibroblast cells in the sample. Fluorescently labeled antibodies are selected based on the flow cytometry data, and the cell mixtures are then sorted using a BD FACSAria cell sorter to purify the cell population(s) of interest. Initially sorted cells may require multiple rounds of sorting depending on the level of purity. Human cells (including both tumor and fibroblast populations) are then expanded and subjected to a further round of fluorescence-activated cell sorting (FACS) analysis to evaluate purity. Clonal populations of cells are then established from the heterogeneous tumor cultures using a soft agar assay.

All purified cultures undergo QC before inclusion in the repository. QC involves FACS analysis, qRT-PCR (for mouse contamination and confirmation that the culture is a tumor or fibroblast), and short tandem repeat analysis (if delinked from the patient) to confirm identity. QC for mixed tumor cell cultures occurs following initial purification at passage 10 and at passage 20.

Tumor cell culture contamination with as little as 0.5–1 percent fibroblast or mouse cells is sufficient for eventual outgrowth of some tumor cultures. Thus, while early passage tumor cultures are frozen back, a representative sample is maintained in culture for 10 and 20 passages for QC analysis. If the sample is noted to contain mouse or fibroblast cells, the sample will then undergo sorting to remove the contaminant and, in some cases, purify the associated human fibroblasts. Since fibroblasts have a finite life span in culture, purified fibroblasts are expanded for no more than five passages

before the final freeze for the repository. A sample of the distribution stock is sent for QC. If the culture fails QC, either the distribution stock sample or the original sample will be sent for sorting and the process repeats. All cultures, tumor or fibroblast, are frozen in the presence of Y-27632 (a ROCK inhibitor, also known as Y compound).

Cultures deemed pure then undergo further characterization for growth with or without the Y compound for proliferation rate, ability to form spheroids, and growth in soft agar to isolate clones. The cultures are tested for rodent or human pathogens, mycoplasma, and sterility. In cases where it is difficult to distinguish a tumor from a fibroblast cell (e.g., sarcomas and mesotheliomas), a qRT-PCR array has been designed to examine cells for a fibroblast signature. All cultures are tested for their tumorigenic potential in vivo by implantation into NSG mice. Information is then added to the PDM repository website and to the certificate of analysis for each sample. To date, there are preliminary certificates of analysis available for four JAX-mixed cell tumor cultures, three clones derived from one JAX tumor line, five mixed tumor cell cultures and 34 fibroblast lines derived from patient material received from NCI participating sites.

A secondary mission of this group is the testing of potential anticancer agents in vivo. Agents are first evaluated in the hollow fiber assay and, if active, are further tested in appropriate human tumor xenograft models in nude or other immunocompromised mice, or in other rodent tumor models. Along with these traditional models, this group also provides support to the Biological Testing Branch's (BTB's) efforts to develop and explore new, potentially more predictive rodent models for assessing experimental chemotherapy regimens.

Significant Achievements

- From September 2015 through July 2016, 1,998 primary models from 1,479 patients were implanted into NSG mice. These specimens originated from the NCI Clinical Center and a variety of participating sites such as CIRB-approved sites, ETCTN, NCORP, U54 sites, and the NCI-sponsored Tissue Procurement Protocol for the Developmental Therapeutics Clinic (# 06-C-0213) and include tumor biopsies, resections, and blood. Additionally, seven glioblastoma multiform samples obtained from an external source were implanted into mice. Material was also received from canine trials, and the group completed the implantation of six canine-derived xenografts. The group also tested PDX models for growth in nu/nu rats implanting 31 models (13 completed and 18 in progress).
- Human tumor samples were initially implanted using the standard subcutaneous route. However, samples have recently been implanted orthotopically. Current orthotopic models include intraprostate, intrapancreatic, intrasplenic, and mammary fat pad implantation.
- The PDM repository currently contains 159 completed tumor tissue models (75 completed this fiscal year) derived from 130 patients from clinical sites and 29 models from material purchased from Jackson Laboratories. Each model contains greater than 200 frozen vials. The models currently represented in the repository include: one breast, 42 digestive/gastrointestinal, four endocrine/neuroendocrine, 23 genitourinary, nine gynecologic, 22 head and neck, 12 musculoskeletal, 27 respiratory/thoracic, 12 skin, two unknown primary, and one hematologic/blood tumor.
- Drug efficacy studies using MPACT agents/combinations (trametinib, everolimus, temozolomide+ABT-888, and MK1775+carboplatin) are in progress in vivo for those PDMs evaluated to have actionable mutations of interest. To date, 45 studies have been completed (20 during this fiscal year), six are in progress, and 13 are in the queue. These studies have now been modified to compare temozolomide and ABT-888 alone and in combination and, similarly, MK1775 and carboplatin alone and in combination.
- A panel of glioblastoma PDX models recently acquired from an external source was identified to be infected with lactate dehydrogenase elevating virus (LDEV), a mouse pathogen that will prevent their utilization in the animal facility. In order to overcome this issue, a FACSAria-based cell sorting procedure has been developed to remove all trace of mouse cells from the tumor. Given that LDEV does not replicate in the human tumor, it is hoped that this procedure will ultimately permit these PDX models to enter into general usage.
- To date, nine mixed cell cultures and 34 fibroblast cell lines have been deposited into the repository. A total of 183 additional mixed cell cultures (91 new this year) and 312 fibroblast (109 new this year) lines are at various stages of characterization and QC. This year, 311 tumorigenicity studies are either in progress or have been completed. There are now a total of 28 pairs of tumor and fibroblast cultures originating from the same patient material with one pair deposited into the repository.
- Given that some patient samples (e.g., head and neck) may be contaminated with mycoplasma, all samples are now tested in house for mycoplasma using the MycoSensor QPCR assay kit. The mycoplasma status of the distribution lot samples is also confirmed by IDEXX Radil.
- Efforts were made to increase the throughput of the FibroQ array analysis (designed to confirm samples contain either pure fibroblasts or pure tumor). The array was modified to focus only on those transcripts that were the best identifiers of fibroblasts (e.g., GREM1, FN1, and Collagens) or tumor cells (e.g., cytokeratins, EPCAM, CD24, etc.) resulting in a fourfold increase in throughput.

- Completed development of the 'Surface scan array' – a qRT-PCR array designed to discover expression of antibody-targetable cell surface. A final list of appropriate targets has been selected and primers synthesized.
- A total of 2,566 CTC samples isolated from blood (via Oncoquick or a filtration method) have now been implanted into mice (1,376 samples during this fiscal year). The transition from EDTA-containing to heparin-containing blood collection tubes has improved success rates to the extent that 10 samples have grown successfully in vivo (five have pathology confirmed and the remaining five are awaiting diagnosis confirmation).
- Recently started to prepare cancer organoids from PDX tumor tissue. The organoids were initially limited to colon cancer to allow for methodology development: initiation of cultures, maintenance, propagation, freezing, and evaluation of the cultures. Different basement membrane and media components were evaluated. Organoid cultures have been expanded to include SCLC, prostate, pancreas, and breast cancer. The group has successfully set up 16 colon, three colorectal, two rectal adenocarcinoma, one small intestine adenocarcinoma, one prostate, and one anal cancer models. Five models have been injected into mice, and all five were tumorigenic. Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) has been performed on one colon model, and preliminary results are consistent with colon cancer. IHC shows the presence of only human cells, and no fibroblasts are present. Further studies are in progress.
- The difficulty in growing CTCs in vivo and in vitro prompted studies to evaluate growth as organoid cultures. CTCs were isolated from blood obtained from colon cancer patients using the OncoQuick methodology, and the cells were implanted into basement membrane extract and treated similarly to the colon organoids derived from PDX models. The number of organoids that form varies from patient to patient. In contrast to the organoids derived from PDX models, growth is very slow and also varies from sample to sample. The CTC organoid culture models have recently been expanded to include pancreas, prostate, SCLC, and breast. There are 16 colon cancer, one colorectal cancer, two SCLC, and six prostate cancers in progress.
- Studies using 31 distinct tumor xenograft models were conducted to assess the antitumor activity of synthetic compounds, natural product extracts, and drug combinations.
- Performed in vivo studies for multiple pharmaco-dynamic experiments (eight performed during this reporting period) assessing the impact of several clinical and experimental agents on a variety of targets of interest to the clinical translation program. Performed 18 studies for the pharmacokinetic group.

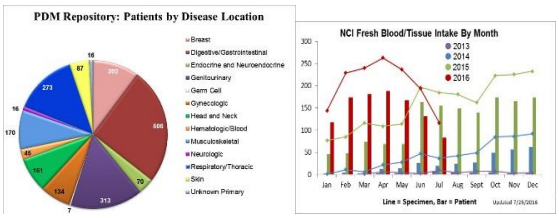
NCI Patient-Derived Models Repository

The overarching goal of the Patient-Derived Models (PDM) Repository is to develop more than 2,000 unique PDMs, including both patient-derived xenografts (PDXs) and in vitro patient-derived cell cultures (PDCs; tumor cultures and cancer-associated fibroblasts). Ideally, each common disease will be represented by 75–100 unique PDX models, representing the genetic landscape of that disease, to allow for in-depth molecular comparisons and efficacy studies. Starting material for model creation includes patient biopsies/resections and circulating tumor cells (CTCs) isolated from blood samples using ApoStream technology (PADIS), and novel techniques developed in the BTB. The repository will distribute all PDX and PDC models through a publicly available website, along with DNA, RNA, and protein pellets. The website will house a public-facing PDM database interface that will include extensive molecular characterization information, patient clinical history, and patient social history for all models. A key component of this repository is to make a set of standard operating procedures for all aspects of PDM creation, propagation, and quality control available to the public.

SIGNIFICANT ACHIEVEMENTS

- The repository conducts bi-weekly operational meetings with NCI senior management, the Biological Testing Branch (BTB), the Molecular Pharmacology Branch (MPB), the Biometrics Research Program (BRP), the Small Animal Imaging Program (SAIP), and the Molecular Characterization Laboratory (MoCha) to discuss specimen tracking and analysis, coordinate preclinical trials using the models, and review progress towards creation of both PDXs and PDCs. To date, the repository has received 3,887 specimens from 2,791 patients (Figure 1A, Table 1).
- The repository receives specimens through a Central Institutional Review Board (CIRB) Tissue Procurement Protocol (9846) and a DTC Tissue Procurement Protocol (06-C-0213). Overall, a total of 15 Comprehensive Cancer Centers and 2 intramural clinics are recruiting patients through the 06-C-0213 protocol, and 23 lead academic organizations with over 140 clinical centers are recruiting patients in the 9846 protocol. All centers are funded through NCI Administrative Supplements. Progress towards these supplements are tracked by the PDM staff and reported to NCI's Office of Grants Administration.
- The first five CTC-derived PDXs have been confirmed by pathology with several more pending analyses. Efforts continue to establish best practices and feasibility studies for creating PDXs from 24-hour old patient blood draws. Preliminary studies growing enriched CTCs as organoids before implantation into mice show promise.

- The repository has a defined PDC workflow with key go/no-go definitions along the model development pathway; these same practices are being applied to organoid culture development. The PDC master tracking sheet established last year continues to provide transparency of progress to NCI as well as allow identification of models where paired PDX and in vitro cultures exist.
- The repository has completed 46 PDX drug studies to date including on-going efforts to pre-clinically model the NCI-Molecular Profiling-Based Assignment of Cancer Therapy (NCI-MPACT) Clinical Trial. The repository is currently developing plans with NCI to test standard of care and agents of interest on a panel of PDX models for a feasibility study of drug response in PDX models.



(left) Representation of cancer diagnoses, divided by body location, that have been brought into the NCI PDM Repository. Numbers represent the total number of patients with the given diagnoses that have consented to provide specimens. (right) The line graph indicates the number of specimens (biopsy, resections, and blood) received monthly into the PDM Repository; bar graphs represent the corresponding number of patients enrolled. Both blood and tumor specimens can be collected from patients as part of the tissue procurement protocols.

	# Specimens	# Patients
Overall	3,887	2,791
Since 9/1/2015	2,469	1,834

Patient enrollment and specimen (biopsy, resections, and blood) intake into the PDM Repository as of 7/23/2016.

Tumor and Natural Products Repository

In October 2014, the management of operations within the Tumor and Natural Products Repository was transitioned to Leidos Biomed. The repository houses transplantable in vivo–derived tumors and in vitro–established tumor cell lines from the various species in low temperature storage. It also houses a collection of natural products NCI has acquired from over 25 tropical and subtropical countries worldwide. Over the past year, the repository has successfully provided necessary services to NCI including completion of approximately 7,000 slow rate freezes consisting of approximately 90,000 vials; sending approximately 1,900 shipments totaling approximately 23,000 samples; and receiving approximately 70,000 tumors and approximately 17,500 natural products samples for storage.

Pharmacokinetics Laboratory

The Pharmacokinetics (PK) Laboratory supports the Office of the Associate Director, Developmental Therapeutics Program (DTP), and is responsible for providing information related to the preclinical chemistry and pharmacology of new anticancer agents identified by DTP. This information is then applied to developing methods for drug analysis that are used in the PK Lab to analyze samples from Phase 0 and Phase 1 clinical studies. Many of these clinical studies are first-in-human studies.

The analysis of drug candidates in biological matrices involves creating, applying, and validating methodologies for each drug candidate. Such efforts require extensive and specialized knowledge of analytical techniques (i.e., HPLC, LC-MS, LC-MS/MS, and LC-HRMS), and biological techniques that encompass the metabolic processes acting on the drug candidate.

Significant Achievements

- *TdCyd (NSC 764274):* The laboratory continued to improve and adapt methods of monitoring TdCyd and metabolites from a variety of sample matrices, including plasma, cell media, and DNA. These methods were used to provide analytical support to various DTP branches/labs, including the following: (1) BTB, (2) PADIS, (3) the Drug Synthesis & Chemistry Branch (DSCB), and (4) Toxicology & Pharmacology Branch (TPB). Results were reported at working group meetings and used to support the IND filing of TdCyd for use in human trials. A Phase 1 clinical trial is underway, and the PK Lab is responsible for analysis of TdCyd and metabolites in plasma and urine samples. In addition to PK analysis and metabolism research, the laboratory conducted extensive cell studies on as many as 12 different cell lines, which provided information about optimizing doses, time of exposure, and toxicity of metabolites.
- *Methoxyamine and temozolamide combination clinical trial (NSC 3801 and 362856):* The laboratory analyzed patient plasma and urine samples through eight dose escalations.
- *Paclitaxel and nilotinib combination clinical trial (NSC 125973/747599):* The laboratory set up methods of analysis for paclitaxel and its major metabolites and nilotinib.
- *Aza-TdC(NSC777586):* The laboratory developed LC-HRMS methods of analysis for Aza-TdC, its major degradation product and its major metabolite. An HILIC-LC/MS method has been used to analyze Aza-TdC plasma levels in mouse, rat, and dog studies, resulting in the discovery of good oral absorption and a lack of significant circulating metabolites. A RP-LC/MS method of analysis is being applied to determine Aza-TdC incorporation into DNA. Results from these studies have been presented to team working groups in order to support eventual IND filing.

- *IPdR(NSC726188):* Using methods developed in the laboratory, patient plasma samples from a newly initiated Phase 1 clinical study are being analyzed. Parent drug, active drug, and metabolites are being measured.

Drug Chemistry Group

The primary objective of the Drug Chemistry Group was to provide the necessary medicinal and synthetic chemistry knowledge, expertise, and technical skill to support NCI's NExT Program as directed by the Division of Cancer Treatment and Diagnostics (DCTD), Developmental Therapeutics Program (DTP), and the Drug Synthesis & Chemistry Branch (DSCB). Due to staffing reductions in this laboratory, the work previously assigned to this lab became the responsibility of NCI in October 2015.

Investigative Toxicology Laboratory

The mission of the Investigative Toxicology Laboratory (ITL) is to develop and implement mechanism-based in vitro models to identify potential liabilities and investigate mechanisms of target organ toxicities in support of programs within the NCI-DCTD. In FY2016, the ITL continued scientific qualification of new assays, protocol optimization of established assays, and use of these assays in support of NExT projects.

SIGNIFICANT ACHIEVEMENTS

- *Characterization of Mcl-1 in regulation of contractile function and viability of human-induced pluripotent stem cell (hiPSC)–derived cardiomyocytes:* Chemotherapeutics targeting the bcl-2 family member, Myeloid cell leukemia sequence 1 (Mcl-1), are being evaluated as potential anticancer therapies due to the role of Mcl-1 in promoting survival of Myc-induced cancer. It has become apparent that both the anti-apoptotic and mitochondrial functions of Mcl-1 play important roles in multiple biological processes. To evaluate whether the hiPSC-cardiomyocytes can be used to elucidate on- or off-target toxicity, ITL conducted investigations to characterize the role of Mcl-1 in maintaining contractile function and structural integrity of hiPSC-derived cardiomyocytes in vitro. Knocking-down Mcl-1 proteins by over 70% with Mcl-1 siRNAs was associated with a sub-lethal injury in cultured hiPSC-cardiomyocytes, demonstrated by activation of Caspase 3/7 activity, Caspase-3 cleavage, minimal LDH release, and changes in cardiac myocyte activities such as increased spontaneous beat rate, decreased beating amplitude and reduction in cellular impedance. Mcl-1 knockdown also resulted in degenerative ultrastructural changes in mitochondria, nuclei, sarcomeres and increased appearance of autophagic vesicles. Further, Mcl-1 knockdown potentiated doxorubicin-induced cardiomyocyte injury. In addition, removal of serum from the culture media potentiated Mcl-1 knockdown-induced cell injury. These results provide a knowledge base for use of this in vitro system as an initial screen for Mcl-1 inhibitors.
- *Support to Mcl-1 project:* Using multiple analytic platforms, ITL conducted cardiotoxicity profiling for discovery Mcl-1 inhibitors and two positive controls (sabutoclax and UMI-77). ITL also performed image analysis with Definiens to quantify the selected target area of interests in mouse spleens from a tumor xenograft study with a Mcl-1 inhibitor.
- *Characterization of hiPSC-cardiomyocyte (CM) maturation:* Use of hiPSC-CMs in cardiotoxicity evaluations during drug discovery has increased. Use of hiPSC-CMs with variable states may confound in vivo extrapolation (or recapitulation) of effects. hiPSC-CMs were cultured for two weeks up to more than six months and the state of maturation assessed by mRNA expression, protein expression, subcellular ultrastructure, electrophysiology, and contractile function, with the use of multiple analytic platforms including RNA microarray, Capillary Western Blot (WES), Transmission Electron Microscopy (TEM), High-Content Image Analysis (HCA), Microelectrode Array (MEA) and Impedance (xCELLigence Cardio). ITL compared the responses of these matured hiPSC-cardiomyocytes to less mature two-week cultures. hiPSc-CMs developed a more mature and adult-like phenotype when time in culture is increased. Indicators of advancing maturity were continuous changes in subcellular organelles (mitochondria, sarcomeres and autophagosomes, etc.), increases in mRNA or protein expression of factors that regulate Ca2+ cycling, adrenergic activation and critical regulators of cardiac electrophysiology. Importantly, remarkable changes in responses to specific modulators of major cardiac ion channels (hERG, Cav1.2, Nav1.5, KVLQT1, and HCN2/4) were noted. These results suggest that consideration of the use of matured hiPSC-CMs in cardiac liability screening is warranted.
- *Optimization and implementation of immuno-precipitation (IP) as a protein-protein interaction evaluation technique:* To evaluate protein-protein interactions (i.e., Mcl-1 with its binding partners), ITL has established an IP technique that uses the Dynabeads system. Bak was identified as the primary binding partner of Mcl-1 in hiPSC-cardiomyocytes, which is followed by Bim. This Mcl-1-Bak IP assay is being used to assess the effect of Mcl-1 inhibitors on this important interaction.
- *Improved Rat DRG culture assay:* ITL conducted additional experiments to quantify the expression level of marker proteins Tuj-1, vimentin, SOX2, and glutamine synthetase (GS) by capillary electrophoresis, and confirmed the presence of SOX2, mainly in nuclei of Schwann cells, and GS in both neuronal and non-neuronal cell cytosol.

- *Contribution to the CiPA initiative:* Comprehensive In Vitro Proarrhythmia Initiative (CiPA) is a multisector initiative with the goal of developing a new in vitro paradigm for cardiac safety evaluation of drugs that provides a more accurate and comprehensive, mechanistic-based assessment of proarrhythmic risk. ITL leaders serve on the Health and Environmental Science Institute (HESI) Technical Committee on Cardiac Safety. Specifically, ITL co-authored a manuscript with the data collected in the pilot study of the CiPA initiative and continued to play a key role in the Phase II validation study for study design and to blind-code and distribute the test compounds to all participating sites with support from NCI Drug Synthesis & Chemistry Branch and Chemotherapeutic Agents Repository group.
- *Support of NExT project teams:* ITL continued the mechanistic study of Silvestrol pulmonary toxicity with the human and canine precision-cut lung slices.
- *Support of the 5-Aza-TdCyd project:* ITL evaluated the bone marrow toxicity of 5-Aza-TdCyd (777586), along with two other nucleoside analogues Decitabine (127716), 5-Azacytidine (102816) and the positive control compound topotecan in our Human CD34+ assay and results reported to the project team.
- *Support of the Cardiac glycoside project:* Cardiac glycosides are currently being evaluated for anti-cancer effects. ITL conducted cardiac safety profiling with hiPSC-cardiomyocyte Impedance and MEA assay on seven cardiac glycoside analogues and monitored the ion-channel panel study in Charles River Laboratory/ChanTest. These evaluations are ongoing.

Information Technology Support

The DTP Computer Center (DTPCC) provides computer support, including operations, technical support services, and software development, to DTP and DCTD. DTPCC provides core services for many aspects of DTP's information systems requirements, including data acquisition within the laboratories, data analysis, and web publication of experimental results. Computer support is provided for many functions of the DTP including: identifying and scheduling compounds to test, preparing and handling the compounds, performing the experiments, and analyzing experimental results for activity.

Applications servers hosted on the Microsoft Windows Server platform support the planning, analysis, and data storage tasks of the program. Windows servers are utilized as application development and deployment platforms, and, through web-based services, provide researchers with an interface to the DTP data and tool repository.

DTPCC support includes the facility management of a modular building to house the computer systems (Building 378) and the administration of 129 servers. Operational support consists of maintaining all server platforms at the highest level of availability and performance. This includes performing system upgrades, backup and recovery operations, diagnosing and resolving problems, and implementing and monitoring system security features. All system operations are completed utilizing practices that minimize system downtime and the impact on a global research community. System consultation and support to the various software developers and scientists who use the system are also provided.

Core applications supported by DTPCC staff include both those developed by the DTP in-house and commercial-off-the-shelf (COTS) data management and analytical applications. Among those applications are the ORACLE RDBMS, Biovia Pipeline Pilot, CambridgeSoft ChemBioOffice Enterprise, Microsoft SharePoint, StudyDirector, Accord, Spotfire, Certara D360, Apex, and Laserfiche. Operating system administration support includes Windows (2003, 2008, 2012, Hyper-V) and Linux.

The Windows Server Support Group provides support for Oracle, Windows, and Linux-based servers. The primary responsibilities in this area are applications and data storage management, performance tuning, data security, and RDBMS availability.

The Web Application Development Group is responsible for the creation and maintenance of program web pages and applications, as well as Java and Oracle development activities.

Significant Achievements

- DTPCC developers created a Pipeline Pilot protocol that allows a user to generate a customized report with several kinds of information about a set of extracts. The user can specify the extracts, which report sections should be included, and whether to include all samples or only those in a particular building. It used to be necessary to generate three separate reports to obtain the same information.
    - A protocol was developed that allows a user to specify a set of plate and well combinations, or entire plates, and generate a report describing the contents of each plate well. The user can specify which of 17 optional columns will be included in the report.
    - Another protocol customized Biovia's Chemical Registration software for the Drug Information System (DIS). This enables the Drug Synthesis and Chemistry Branch (DSCB) to use Chemical Registration to register new compounds and record the receipt of material for compounds.
- Migrated CambridgeSoft applications from Frederick to CBIIT. The operating system platform was upgraded from Windows Server 2003 to Windows Server 2012. The supporting Oracle database was upgraded from version 10G to 11G. CambridgeSoft was upgraded from version 12 to version 15. Because of major changes within the product, migration was extremely complex and required close and extensive collaboration between the DCTD IT team and CambridgeSoft software engineers. This achievement provided DCTD with new features available in both Oracle 11G and the new CambridgeSoft suite.

- Completed the migration of nine Windows 2003 servers to Windows 2008 or 2012, improving system responsiveness and complying with NIH Security requirements.
- PL/SQL packages were written, enabling the transfer of data from Chemical Registration to legacy tables.
- Java routines were implemented that use web services to extract in vivo data from the new Studylog 3 system for use in reporting supplier reports and graphs of test results.
- Migrated the Natural Products Repository Support System (NPRSS) from an Oracle Application Server (OAS) server to an Oracle Fusion–based server. Oracle forms were redesigned in Java, allowing for enhanced tracking of shipments.
- Finished NPRSS modifications, allowing the input of thousands of Pre-Fraction compounds into NPRSS.
- Built and deployed a three-tiered application platform to support Chemical Registration, Insight, and Pipeline Pilot.
- Took over the web design, content management, and maintenance of the Office of Cancer Complementary and Alternative Medicine (OCCAM), cam.cancer.gov.

Software Licensing Support

At the request of DCTD, DTPCC coordinated the purchase of licenses and maintenance contracts for 13 groups within DCTD; 117 software packages were included. Additionally, the group completed an HHS-OIG–mandated audit of all software (approximately 17,000 items) on all DTPCC servers.

Computational Drug Development Group

Through the application of computational data modeling, including bioinformatics, chemoinformatics, molecular visualization, computational chemistry, molecular modeling, structure-based medicinal chemistry, and statistical data mining, the DTP Computational Drug Development Group (CDDG) supports the mission of the BEC decision process, the NeXT program, and the CBC program.

Significant Achievements

p97 Structure-Based Modeling and Data Mining

- Upon request, assisted the University of Pittsburgh Chemical Diversity Center (Professors Peter Wipf and Donna Huryn) with understanding their available VCP/p97 SAR through the use of detailed all-atom models. Prior to CDDG involvement, they were not satisfied with the available binding models, due to inadequate basis for explanation of the activities of more than 500 inhibitors produced for the CBC (triazole and phenyl-indole series).
- Based on existing X-ray and EM structure models plus inhibitor activities, refined the binding site within experimental resolution, providing structural explanations for all of the observed indole SAR. Refined models for triazole analogs have also been completed.
- Models with sufficient detail are now available for design of new inhibitor analogs.
- Informatics identified potential natural product extracts and an investigational drug (BAY-87-2243) that may help refine ADMET issues for triazole analogs.

KDM5A, KDM5B, and KDM5C analog evaluation in structure-based models

- Using leads for the KDM5A/B target, the CDDG provided structure-based support via computational modeling with NCATS Synthetic Chemistry Group.
- CDDG predicted requirement for Zn++ binding sites for activity of KDM5 enzymes, confirmed by Emory.
- All current SAR from prior-art compounds was rationalized with detailed all-atom models of KDM5A, KDM5B, and KDM5C. Additional patent literature SAR was accounted for based on refined models.
- Multiple predictions from other modeling efforts have been proven invalid.
- Validation of CDDG binding models were confirmed with X-ray co-crystals and activity data.

Computational support for pre-clinical drug candidates

- Employed computational methods (e.g., modeling and data integration) to support like compounds that are strong pre-clinical drug candidates.

Support Provided by the Clinical Monitoring Research Program

The overarching mission of the Division of Cancer Treatment and Diagnosis (DCTD), Developmental Therapeutics Branch (DTB), is to evaluate innovative anticancer compounds in early phase clinical trials, while providing outstanding clinical care for patients with different cancers. An important focus is first-in-human clinical trials, particularly those that incorporate pharmacodynamic and pharmacokinetic endpoints, with the goal of informing subsequent clinical development. CMRP provides one senior nurse practitioner and three clinical research nurses to support these efforts.

CMRP staff supports 22 ongoing protocols and, more specifically, independently coordinates every aspect of the research efforts for these protocols. Aside from patient recruitment efforts, a clinical research nurse reviews patient referral records, contacts potential research patients to evaluate their present health status, schedules patients for screening clinic visits, and coordinates the patients' visits once they arrive at the National Institutes of Health (NIH). The

senior nurse practitioner evaluates patients on all 22 protocols and takes care of their day-to-day medical care needs.

During FY2016, CMRP staff members assisted in initiating and implementing three new protocols: (1) 16-C-0109 Phase I Trial of the Combination of the Heat Shock Protein-90 (HSP90) Inhibitor Onalespib (AT13387) and the Cyclin-Dependent Kinase (CDK) Inhibitor AT7519 in Patients with Advanced Solid Tumors; (2) 16-C-0087 Phase I Study of Veliparib (ABT-888), an Oral PARP Inhibitor, and VX-970, an ATR Inhibitor, in Combination with Cisplatin in Patients with Refractory Solid Tumors; and (3) 15-C-0200 Molecular Analysis for Therapy Choice (MATCH) (EAY131). Additionally, CMRP staff has assisted in screening more than 110 patients for protocols and, so far, has enrolled 91 patients on the team's various protocols.

A CMRP clinical research nurse is independently responsible for coordinating the opening of Cancer Therapy Evaluation Program (CTEP) study 9149 to multiple sites, with an estimate of up to 70 participating sites. One of the clinical research nurses runs the conference calls, and manages the multi-site and regulatory aspects of this trial. Since September 2015, the clinical research nurses successfully completed three sets of protocol audits without any negative findings.

Cancer Imaging Program

Support Provided by the Applied and Developmental Research Directorate

The Cancer Imaging Program (CIP) support included oversight of radiopharmaceutical chemistry, medical imaging agent availability, regulatory affairs, clinical trials quality assurance support, and imaging bioinformatics. Oversight of critical research subcontracts awarded to facilitate both preclinical and clinical research activities for imaging agents, as well as the specific radiochemistry management support provided to the radiopharmaceutical facilities in Frederick, has served to ensure wider availability of investigational agents for exploratory and Phase I–III clinical trials. Regulatory support has been focused on managing the life cycle of the CIP INDs, assisting with the advanced development of the CIP- and non-CIP-sponsored investigational imaging agents, supporting intramural and extramural clinical trials, and providing access to CIP imaging agents for additional researchers via several cross-reference mechanisms that have been implemented. Oversight of imaging bioinformatics, including the teams' leadership in numerous informatics communities and imaging networking endeavors, has also been a key contribution. These efforts, through managing research subcontracts, providing regulatory and medical affairs expertise, and participating and leading imaging bioinformatics communities, have served to support the mission and goals of CIP for the NIH intramural and extramural research communities as well as high-profile NCI programs such as the NCI Experimental Therapeutics (NExT) Program.

In addition to other NCI programs, CIP staff collaborated with NIH groups at the Clinical Center in Bethesda, MD, and in Frederick, MD; with networks including the Eastern Cooperative Oncology Group-American College of Radiology Imaging Network (ECOG-ACRIN), American College of Radiology (ACR), and Society for Nuclear Medicine and Molecular Imaging (SNMMI); and with other scientific and regulatory organizations, including the FDA. Interagency meetings were conducted with regulatory and medical counterparts in the FDA's Division of Medical Imaging Products. These efforts have supported CIP's goals of promoting the wider use of medical imaging in diagnosis, response to therapy monitoring, therapeutic drug development, and medical decision-making for cancer patients.

During the past nine years, CIP emphasis has shifted toward the development and delivery of a variety of imaging products, requiring new strategies, resources, and external outreach activities. The Phase 0 initiatives and the dissemination of short-lived tracer technology are two prominent examples of initiatives resulting from this shift in CIP focus. The NExT Program integrated the activities of several cross-institute imaging activities into two decision-making committees. CIP continues to be involved in NExT projects by providing support and guidance to the Radiopharmaceutical Chemistry Group and operating the Leidos Biomed Radiopharmacy. Current efforts have been maintained to support NCI DCTD through its Scientific Advisory Committee (F-alpha-methyl-tyrosine, F-AMT [DTFFAMT] ongoing), and also through the NExT Program (NIR-panitumumab completed and others). F-AMT and NIR-panitumumab were the result of internally developed ideas requested through CIP, while the others were from extramural investigators. Efforts to support researchers in CCR continue by producing F-DHE, a reactive oxygen species tracer, for use in cellular imaging and, if positive, animal models.

The CIP bioinformatics team continues to provide leadership, community management, infrastructure, and tools to support imaging research initiatives, enhance reproducibility in research, and support the NIH big data mission.

CIP continues to manage and expand The Cancer Imaging Archive (TCIA) research subcontract, which it leverages to provide support to the public clinical imaging research community, the CIP Quantitative Imaging Network (26 U01 grantee institutions), and four multi-institutional research groups focused on the novel field of imaging genomics. CIP leads the development of new technologies and methodologies for integrative data analysis, clinical imaging data de-identification and curation, and image processing, and has also provided leadership in developing organization and logistics for grand challenges designed to compare the performance of image analysis software.

Radiopharmaceutical Chemistry and Imaging Agent Research Subcontract Support

CIP's radiochemistry program primarily supports the goals of the Imaging Drug Group, providing development of new imaging agents and follow-up testing of currently administered agents. This groundbreaking work may lead to increased availability of types of agents for clinical trials. Maturation of this effort is documented by the fact that the original space designated for this work was transitioned to a United States Pharmacopeia (USP)–level radiopharmacy capable of delivering clinical-grade human doses for preclinical and clinical evaluation efforts by a certified nuclear pharmacist.

To date, the radiopharmacy has supplied 18 doses of [18F]-fluoroestradiol for administration to 11 subjects at the Clinical Center in Bethesda, MD under CIP's IND; enrolled its first three subjects for 89Zr-panitumumab; and has enrolled 99 subjects for DCFBC, a radiotracer for prostate cancer which has proven so successful that the number of overall doses was appended from its original target of 45 to a new total of 125. During FY2015, the radiopharmacy was relocated to an alternative site in Frederick, MD. Planning meetings were successful, and newly occupied space (Building 459) has better infrastructure (former GMP space) and is much larger than the previously used space. The new facility has been operating smoothly since July 2015. A NIH Red Team assessment of the radiopharmacy confirmed operations were compliant with CFR and USP regulations, allowing them to continue to supply products to the NIH Clinical Center and leading to additional clinical trial opportunities.

Research subcontracts with extramural sites were coordinated to facilitate the formal clinical trials performed at the CIP Phase I and II NCI contract sites. In addition, efforts to make promising radiopharmaceutical agents available to the research community for clinical investigation have been significantly broadened. Because the PET tracers have no intellectual property associated with them, commercial entity investment is viewed as risky. CIP personnel were involved in negotiations with the three major suppliers of cyclotron-produced isotopes and radiopharmaceuticals for implementing fluoro-L-thymidine (FLT) tracer synthesis and applying for a DMF so the tracer could be supplied to NCI trials.

CIP provides selected PET radiopharmaceuticals to support both small early-phase trials as well as larger trials. Through a research subcontract with the University of Pennsylvania, 5-fluoro-2'-deoxcytidine (5FdC) is the only additional tracer remaining to be investigated. Radiolabeled 5FdC has been identified as an imaging agent of special interest to DCTD as part of its Phase 0 Clinical Trial #8865 involving 5FdC with tetrahydrouridine (THU) for the treatment of head and neck, lung, bladder, and breast cancers. The drugs 5FdC and THU are being used in a cancer treatment study. Since researchers continue to investigate how 5FdC works in the body, researchers are assessing a modified form (radiolabeled 18F-5dC) using imaging studies to see how the drug reacts with the cancer. A non-severable research subcontract was awarded to the University of Pennsylvania to provide 50 doses for 25 patients over the next four years.

Regulatory Affairs Support

Comprehensive regulatory support was provided for CIP activities related to the IND and New Drug Application (NDA) development process for imaging agents. CIP facilitates the development of promising diagnostic agents, many of which are PET drugs that fall under special FDA oversight and regulation because their manufacturing often poses unique challenges and they may not be able to undergo the same amount of standard preclinical testing or early-phase clinical testing, as is required for more conventional drug development. A variety of regulatory mechanisms and strategies must therefore be kept in place at CIP as part of the life cycle of the CIP-sponsored imaging agents and to enable others to cross-reference this information for their own research.

The following eight CIP-sponsored INDs are currently managed and supported by the Regulatory Affairs staff:

1. IND 71,260 ([18F]-fluoro-L-thymidine), a proliferation agent
2. IND 68,556 (ferumoxytol), a blood-pool MR agent
3. IND 76,042 ([18F]-fluoromisonidazole), a hypoxia agent
4. IND 79,005 ([18F]-fluoroestradiol), an estrogen receptor agent
5. IND 103,429 [18F]-NaF, a bone-scanning agent
6. IND 116,229 [89Zr]-panitumumab
7. IND 122,503 [18F]-DCFBC, a prostate-specific membrane-antigen agent
8. IND 70,651 hyperpolarized pyruvate (^{13}C) injection, an MRI agent

Two INDs and one NDA were closed during the current year as demand for those agents waned.

Multiple protocols are being conducted under each of the CIP-sponsored INDs that range from Phase 0 to Phase III, with Phase II having the most protocols and all having differing regulatory requirements. Nine clinical trials were active and enrolling patients during this reporting period. Many of the trials have inherent regulatory complexities due to the involvement of multiple investigators, sites, local IRBs, and contract organizations located in the U.S., Canada, and other foreign countries. During this reporting period, CIP Regulatory Affairs issued 11 letters of authorization allowing independent researchers to cross-reference the materials in CIP INDs for their trials.

The still relatively new IND for hyperpolarized pyruvate continues to require significant regulatory affairs maintenance. A Letter of Authorization was just issued to CIP to enable cross-filing to the original IND for this agent. CIP staff continue to work closely with federal staff to revise and update the hundreds of regulatory and production materials on the Cancer Tracer Synthesis

Resources pages on the CIP website pertaining to this agent so that the research community has access to this data to pursue their own research.

A new approach developed in 2016 in cooperation with the federal CIP staff is to enable supply of IND Agents to NCI-sponsored trials by skilled academic sites. Investigational drugs require an IND from the FDA to test in humans and, given the radiological half-life of these agents is short (commonly 110 minutes), drugs must be produced in close proximity to the patient and administered expeditiously. Commercial companies that used to provide such PET radiopharmaceuticals are not covering the requested supply any longer; therefore, CIP is recruiting these skilled academic sites to fill the void for the ECOG-ACRIN cooperative group's and NCI Early Treatment Clinical Trials Network (ETCTN) grant program's clinical trials. NCI requires that NCI sponsor the IND for its multicenter trials and, therefore, must certify the chemistry and manufacturing controls for each selected site meet NCI's specification for each drug and manufacturing site. The Medical Affairs Scientist reviews the requested application from each site and makes a recommendation to the CIP Associate Director to allow or deny said application to produce the agent. The CMC for every site for the specific must be filed with FDA (DMF or IND).

- The agent must be "equivalent" across sites
- The agent must meet the NCI specifications
- The site must comply with USP<823> or cGMP
- The site must be experienced
- NCI not responsible for site CMC compliance/filings.

Under this format, six sites have been approved for FLT production, three for FES production, and one for FMISO production (several others have applied but did not meet the requirements). This effort will continue on an ongoing basis as more applications are received.

Medical Affairs and Quality Assurance Support

The CIP support team works with NCI CIP medical officers, the NCI CIP Clinical Trials Branch (CTB) chief, the Eastern Cooperative Oncology Group-American College of Radiology Imaging Network (ECOG-ACRIN) program director, and the ECOG-ACRIN senior staff to provide day-to-day oversight for the ECOG-ACRIN QA, monitoring, and audit programs. ECOG-ACRIN operational (QA monitoring and audit) reports are reviewed primarily by the CIP Regulatory and QA support team. Audit tracking schedules are reviewed in advance and evaluated against actual audit performance. Preliminary audit reports are sent to the Regulatory and QA support team for review immediately post-audit, thus providing an early warning opportunity. Should prompt action be necessary, the CIP Regulatory and QA support team work hand-in-hand with NCI and ECOG-ACRIN to define and implement a course of timely intervention to mitigate suboptimal site performance. Items requiring immediate action are brought to the attention of the ECOG-ACRIN program director and the CTB chief. Strategic support is still provided to ECOG-ACRIN to assist in improving regulatory, IRB, and protocol compliance.

During the reporting period, staff supported upgrades to systems and processes for the receipt and tracking of adverse events (AEs) and auditing reports at CIP for the continuing ECOG-ACRIN legacy trials. CIP staff completed modifications and evaluations of existing AE reporting systems designed for therapeutics, so that they now meet the needs of imaging clinical trials. Additional regulatory projects included: (1) ongoing co-monitoring of specific trial sites within ECOG-ACRIN to obtain information necessary to conduct a comprehensive process audit of the cooperative group; (2) supporting amendments to the cooperative group guidelines so that ECOG-ACRIN could be managed under the same policies as the other cooperative trial groups; and (3) supporting the convergence of the National Clinical Trials Network (NCTN) and the Clinical Trials Monitoring Branch (CTMB) guidelines, and benchmarking ECOG-ACRIN to standardize imaging standard operating procedures.

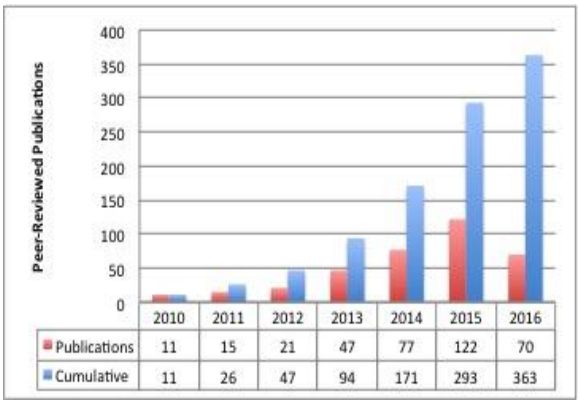
The clinical trials administrator continued to work collaboratively with the CIP ECOG-ACRIN program director and medical officer, and other subcommittee/ working group/project members, to provide clinical insight and guidance to ensure that imaging-related content was included in NCI's processes, procedures, and working guidelines. These groups included the Clinical Trials Reporting Program, CTMB, NCTN, the Investigational Drug Branch/protocol review, the Cancer Trials Support Unit, the ECOG-ACRIN QA committee, and CTEP AE committees. Due to all AE monitoring and reporting switching over to CTEP, members of the CIP support staff are no longer required to support this effort.

The Clinical Trials Administrator also continued to support the CTB by monitoring all Clinical Data Update Systems (CDUS) and Adverse Event Expedited Reporting Systems (AdEERSs) reporting for CIP imaging protocols. CDUS reports were compiled for review by the medical officer on a monthly basis. The reports were then presented quarterly at the CTB meetings. Many variables were regularly monitored for trend analyses, patient safety, and International Conference on Harmonization/ Good Clinical Practices (ICH/GCP) compliance, including treatment start date, monitoring method, imaging agent or Network Steering Committee (NSC) lead agent, protocol number, patient ID, institution, course ID, last changed date in the system, outcome grade, and AE toxicity. In addition, the AdEERS report (a reporting system for expedited and serious adverse events) was consistently monitored for such variables as site protocol compliance trend analyses, patient safety, ICH/GCP compliance, imaging agent protocol number, IND type, protocol name, patient identification, institution, NCI receive date, AE description and start date, imaging agent, attribution outcome, ticket number, and amendment. Each event was reviewed weekly with the medical officer and presented at the monthly CTB meeting. This information remains useful, in conjunction

with annual reports, when filing NCI imaging INDs, continuing reviews for the Clinical Center IRB, and working on CTB ad-hoc projects.

Imaging Scientific Support

The Cancer Imaging Archive (TCIA): Managed the TCIA project, including conducting monthly NCI Advisory Group meetings that set scientific priorities for new data collection and technological development activities. Major new collections were processed including the Ivy GAP GBM, multiple myeloma, focused ultrasound, and NSCLC radiomics. Over 67 datasets now reside in the archive with 360 peer-reviewed publications now based upon TCIA-hosted data (see figure below). Staff conducted a full analysis of implementation costs for TCIA (hosting, curation stages, collection, etc.) and developed a coordination strategy with CBIIT removing redundancy and improving efficient use of resources overall. Additionally, staff managed the transition of the TCIA contract to a new contractor site.



	2010	2011	2012	2013	2014	2015	2016
Publications	11	15	21	47	77	122	70
Cumulative	11	26	47	94	171	293	363

CIP support program staff directed the development of new digital object identifier (Data DOI) technology so that TCIA is now "publishing" all datasets. New features also allow users to publish derived data to enrich the value of the primary data and also facilitate reproducible research methods. A paper publishing the non- the Cancer Genome Atlas (TCGA) linked data sets was submitted to the journal Nature Scientific Data. The TCIA website was re-designed into data collection, portal, and analysis centers.

NCI CBIIT imaging informatics: Subject matter expertise was provided to the Clinical and Translational Imaging Informatics Project (CTIIP), including archiving of pathology imaging, integrating challenge technology, and developing infrastructure and standards for co-clinical data sets.

Imaging genomics and imaging proteomics: Staff completed the TCGA-linked data set and continued to support multi-institutional scientific groups. A bladder data set was collected from eight institutions and new analysis was supported. Staff worked with the Clinical Proteomic Tumor Analysis Consortium to develop a strategy for the collection of imaging data to support imaging-proteomic analysis and data collection has begun.

Quantitative Imaging Network: Provided leadership to multiple working groups, drove consensus on annotation and archiving, and managed research and challenge data sets on TCIA. Provided support to new grantees to prioritize data collections and improve submission strategies.

FNIH Coding4Cancer: Completed a major data collection effort to provide additional low-dose CT screening data to the National Lung Screening Trial (NLST) for the Coding4Cancer challenge. Provided technical support for development of the management RFP and award as well as project close-out.

NCI precision medicine trials: Assisted, through the TCIA subcontract, collecting imaging data for the Exceptional Responders activity.

Community outreach: The informatics team made presentations and conducted workshops at the Radiological Society of North America, the Medical Image Computing and Computer Assisted Intervention 2016 conference, and other venues, and continue to grow TCIA's user community through social media with more than 700 followers of TCIA news and updates.

Cancer Therapy Evaluation Program

Support Provided by the Clinical Monitoring Research Program

A CRADA program supports NCI's Cancer Therapy Evaluation Program (CTEP) through funding of correlative studies performed during sponsored clinical trials utilizing CTEP IND agents. This serves the extramural community by supporting critical correlative studies that are aligned with NCI-sponsored clinical trials being conducted under separate agreements between NCI and the clinical trial site. NCI has authorized the use of its CRADA funds to support research agreements that cover the cost of the approved correlative studies. The clinical trial site may receive CRADA funding to cover the costs of conducting correlative study activities including assays and investigational imaging, appropriate and reasonable personnel efforts and supplies, direct patient care costs; specimen collection, processing and shipping; institutional indirect costs; and protocol-mandated patient evaluations and/or sample acquisitions.

During the reporting period, support to CTEP included managing the complex acquisitions process to support the growing portfolio of clinical trials requiring correlative science funding. CMRP staff provided high-level administrative and research subcontract management support to more than 55 agreements awarded to more than 30 institutions and vendors, totaling approximately $922,000—an increase of $378,000 since FY2015.

CMRP staff supported the correlative science agreements (including blanket purchase agreements, basic order agreements, task orders, and research subcontracts) that are sponsored by the following eight drug companies: (1) AstraZeneca, (2) Genentech, (3) GlaxoSmithKline, (4) Takeda (formerly Millennium), (5) Bristol-Myers Squibb, (6) Merck, (7) Novartis, and (8) Pharmacyclics.

Enhanced support was provided to AstraZeneca, Merck, Bristol-Myers Squibb, Takeda, and Novartis with the approval of correlative studies for either new or additional sites supporting CTEP protocols conducted at multiple participating institutions. In December 2015, CTEP presented a unique challenge concerning the collection/shipment of pharmacokinetic (PK) specimens collected during the conduct of CTEP-sponsored trials, previously managed directly by GlaxoSmithKline. After Novartis acquired the two investigational drugs (trametinib and dabrafenib), company policy stated that they could no longer work directly with the participating sites. CMRP staff participated in multiple teleconferences with all stake holders (NCI, Novartis, and Leidos Biomed) to discuss potential strategies. As a result, CMRP staff established an agreement with Therapak and effective processes were implemented to ensure provision of PK kits to accrual sites per established timelines, sample shipment, database entry/tracking, and payment for services to Therapak, the accrual sites and corresponding laboratories. A customized Leidos Biomed process was created/implemented to manage the four vendor payment agreements with Therapak for the management, acquisition, invoicing, and payment of services.

CMRP staff continued to manage the comprehensive communication and information dissemination processes among all program stakeholders, including the CTEP drug monitors, the PIs, and the CTEP Regulatory Affairs Branch that manages the CRADA funding. Staff attends weekly CTEP/Investigational Drug Branch (IDB) meetings, coordinates monthly patient accrual deliverables with CTEP/IDB and participating institutions, and coordinates program teleconferences as needed.

Given the Operational Efficiency Working Group (OEWG) recommendations that were implemented in 2010 to increase the pace at which NCI-sponsored trials are initiated, CMRP staff continues to coordinate activities with the Leidos Biomed Purchasing and Subcontracts departments to outline the most efficient process to solicit work and make awards. An efficient purchase agreement process was further customized with additional cost control measures. Incremental funding of the agreements, along with monthly monitoring of deliverables and quarterly progress reports and invoices, provided consistent oversight that enabled the early identification of underperforming clinical trial sites and resulted in cost savings. A streamlined, collaborative communication and funding process continued to be utilized, enabling the prompt funding of the NCI-approved, CRADA-supported correlative work.

CTEP Precision Medicine Initiative

NCI's CTEP is responsible for coordinating the largest publicly funded oncology clinical trials organization in the world. CTEP currently supports over 900 active trials enrolling 30,000 study participants annually, nearly 400 grants and cooperative agreements, and approximately 100 INDs. CTEP-sponsored research spans phase I–III trials in all cancers and treatment modalities: chemotherapy, immunotherapy, radiation, and surgery. On January 30, 2015, President Barack Obama unveiled details about the Precision Medicine Initiative (PMI), a bold new research effort to revolutionize how we improve health and treat disease. NCI-supported scientists are pursuing new technologies and collaborations and conducting new kinds of clinical trials to help fulfill the promise of precision medicine. CTEP is an integral part of this initiative through its support of several large screening studies including NCI-MATCH, Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial (ALCHEMIST), and Lung Cancer Master Protocol (Lung-MAP).

During FY2016, Leidos Biomed continued subcontracts management support that was initiated in FY2015. A sole-sourced subcontract with CCS Associates, Inc. (CCSA) was executed in October 2016 to support precision medicine clinical trials and provide assistance for the administration of multiple scientific committees to implement, monitor, and educate regarding these PMI trials.

CMRP staff members continue to provide technical project management for the agreement, including coordination of bi-monthly CMRP-led stakeholder calls with CCSA, CTEP, and CMRP wherein expectations, processes, division of labor, costs, and scope of work are evaluated. These calls are vital to ensure clear communications, clarify expectations, and maintain the SOW scope of work. Staff also review monthly reports and invoices that document the high-level support being provided to the PMI that is expected to continue through FY2017.

It is anticipated that there will be 2–3 additional precision medicine clinical trials that will require support at any given time. This potential additional support has been discussed at the bi-monthly stakeholders' calls and during initial budget planning meetings with the customer.

Support Provided by the Molecular Characterization Laboratory

Clinical Activities

The Molecular Characterization Laboratory's (MoCha's) clinical laboratory is Clinical Laboratory Improvement Amendment (CLIA) certified, performing multiple analytically validated next-generation sequencing assays in support of NCI's Division of Cancer Treatment and Diagnosis (DCTD) clinical studies and precision medicine initiatives. To support the clinical trials, MoCha has built a strong group consisting of histotechnologists, molecular biologists, a quality assurance manager, bioinformaticians, a program manager, scientists, and a pathologist. MoCha aims to provide scientific evidence to improve patient outcomes by translating information from the comprehensive characterization of molecular alterations in patients' tumors.

MoCha coordinates activities and manages a Next-Generation Sequencing (NGS) laboratory network that includes external subcontractors (Massachusetts General

Hospital, MD Anderson Cancer Center, and Yale University) and MoCha's CLIA laboratory. This network supports the NGS activities for the NCI-MATCH Clinical Trial (CTEP-EAY131). Three additional laboratories have successfully responded to a recent RFP and will be contracted under BOAs. At a minimum, one of these laboratories will be selected to join the NCI-MATCH Clinical Trial effort. The NCI-MATCH NGS laboratory network will screen tumor biopsies from approximately 5,000 patients by performing the NCI-MATCH assay for enrollment in the clinical trial. MoCha led the effort to design, develop, and validate the NCI-MATCH NGS assays for the NCI-MATCH Clinical Trial using harmonized and locked SOPs. This is the first-ever multi-laboratory effort to validate a complex NGS clinical assay using identical chemistry, the bioinformatics pipeline, and harmonized SOPs. The analytical validation study showed that the NCI-MATCH assay has a sensitivity of 97.4 percent, specificity of 99.9 percent, accuracy of 99.9 percent, and overall concordance of greater than 99.9 percent. The validation report was submitted to the FDA as a part of IND submission for the NCI-MATCH Clinical Trial. A favorable response to the submission was received from the FDA, and the agency deemed that the NCI-MATCH Clinical Trial was safe to proceed. Recently, MoCha led the effort to version the NGS assay, adding targets required for trial support. During this versioning, the assay was moved onto a newer and higher-throughput platform. MoCha added resources, as requested by NCI, to the clinical laboratories this year to meet the demands of the rapid study enrollment. Several members of MoCha received NCI Merit Awards for contributing to the NCI-MATCH Clinical Trial planning.

The MoCha clinical laboratory also supports NCI's Molecular Profiling-based Assignment of Cancer Therapy (MPACT) for Patients with Advanced Solid Tumors Clinical Trial (CTEP Protocol #9149), using a custom next-generation sequencing assay panel. This hypothesis-driven clinical trial will screen approximately 700 patients for enrollment. Plans are being implemented for open enrollment at external Experimental Therapeutics Clinical Trials Network (ETCTN) sites.

MoCha senior leadership has been requested to participate in meetings, such as the AACR-NCI-EORTC International Conference on Molecular Targets and Clinical Therapeutics, the National Institute of Standards and Technology (NIST) Genome in a Bottle, and the NCI Intramural Scientific Investigators Retreat, to share experiences in clinical assay development, bioinformatics pipelines, formalin-fixed paraffin-embedded samples used in NGS assays, and NGS application/assay design in clinical trials and studies.

The director of MoCha serves as one of the principal investigators for both the MPACT and NCI-MATCH clinical trials. In addition, members of MoCha are frequent invitees to FDA/Center for Devices and Radiological Health (CDRH) workshops and roundtables for discussions about precision medicine and genomics. MoCha frequently engages reagent suppliers. These interactions have lead the team to ensure lot integrity and monitor expiration dates and has provided suggestions on kit configurations that would reduce reagent costs by approximately 14 percent and decrease reagent waste. The majority of these efforts were spearheaded by MoCha's research associate III.

Recently, MoCha was requested to build its histology and pathology capacity to analytically validate selected immunoassays in the CLIA environment for patient selection, in support of DCTD clinical trials and programs. MoCha recently moved into a new laboratory dedicated to this effort. The group has begun to perform tissue processing, sectioning, staining, and image capture of tumor specimens.

Research and Development Activities

The Research and Development (R&D) group of MoCha is involved with developing new technologies using next-generation technology and has developed robust research assays to support genomic characterization in many different tissues and bodily fluids. MoCha's research and development (R&D) group is directly involved in NCI's Patient-Derived Model (PDM) project initiated by Dr. James Doroshow, DTCD. MoCha is tasked with genomic characterization of all the patient-derived xenografts by performing RNA sequencing and whole-exome sequencing, and using a variety of related clinical assays to fully characterize PDM models. The MoCha histopathology group provides pathology support to the PDM project through morphological and immunoassay characterization of patient-derived xenografts from DCTD's programs.

The advantage of MoCha having both a CLIA-accredited clinical laboratory and an R&D laboratory is the ability to easily convert research-grade assays into clinical assays for patient care. The group has established many collaborative efforts for understanding the predictive biomarkers and treatment responses (effective/ineffective) from patient tumors. MoCha has interacted with NIST in developing clinical reference and control materials. MoCha has successfully licensed 67 plasmids as reference materials through SeraCare for oncology NGS assays.

NIAID INTRAMURAL

Division of Clinical Research

Support Provided by the Applied and Developmental Research Directorate

AIDS Monitoring Laboratory

The AIDS Monitoring Laboratory (AML) performs sequential studies of immune function in patients with HIV disease or other emerging/re-emerging infectious diseases during treatment with a variety of antiviral and immunomodulatory agents. The results of this work aid in the assessment of the efficacy and mode of action of these agents, as well as determine optimal therapeutic strategies that may lead to restored immune function. AML is certified by the Department of Health and Human Services Centers for Medicare & Medicaid Services (CMS) to perform high-complexity testing on human specimens under the auspices of the Clinical Laboratory Improvement Amendments (CLIA) of 1988. Current CLIA-approved tests include white blood cell count, white blood cell differential, and lymphocyte immunophenotyping.

During FY2016, AML provided comprehensive immunological monitoring for 61 clinical research protocols conducted by NIAID's Division of Clinical Research (DCR). Support of these clinical studies resulted in the processing of 2,626 whole blood specimens; 256 leukapheresis specimens; 11,696 sera specimens; 3,222 plasma specimens; 69 respiratory specimens; 70 urine specimens; 20 tissue biopsies; and 22 cerebral spinal fluids. AML performed 81,415 immune cell phenotype determinations; 8,296 D-dimer assays; 50,964 cytokine measurements; 2,626 complete blood count/cell differentials; and 4 immunomagnetic-bead separations. AML cryopreserved 19,460 vials of patients' peripheral blood mononuclear cells (PBMCs); 15,322 vials of serum; 22,300 vials of plasma; 702 vials of urine; and 184 respiratory tract specimens. AML coordinated 191 shipments of patient specimens to investigators located at the NIH and other domestic and international institutions. Specific efforts are identified below.

- A federal surveyor from the Philadelphia Office of the CMS conducted an on-site recertification inspection of the laboratory on April 13, 2016. CMS regulates all laboratory testing performed on humans in the U.S. through the CLIA, Public Law 100-578. AML was determined to be in compliance with the applicable CLIA regulations found in 42 Code of Federal Regulations, Part 493 (42 C.F.R. § 493). As a result of this inspection, AML retained its certification to perform high-complexity tests on patient specimens.
- In support to Dr. Irini Sereti, NIAID, the AML Clinical Flow Cytometry Group used a custom eight-color immunophenotyping panel to perform a detailed analysis of monocyte subpopulations in blood of patients enrolled in three clinical trials: the PANDORA trial, a natural history study intended to evaluate the incidence, predictors, and pathogenic mechanisms of immune reconstitution inflammatory syndrome (IRIS) in HIV-infected adults; the APHRODITE trial, a phase-I study of CC-11050, a novel anti-inflammatory compound, in HIV-1 infected adults with suppressed viremia on antiretroviral therapy; and the ECSTATIN trial, a study of the effects of statin therapy versus aspirin on immune activation in HIV infected participants. Monocytes have been shown to be relevant in immune activation and in the setting of HIV infection, monocyte phenotypes correlate well with soluble biomarkers of clinical relevance, such as interleukin (IL)-6, CRP, and D-dimer. Monocytes have strong relevance in atherosclerosis and monocyte changes are attractive targets for intervention and for study endpoints. During FY2016, monocyte subpopulations were analyzed in 323 patients.
- In support to Dr. Irini Sereti, NIAID, the AML Clinical Flow Cytometry Group continued to examine programmed death-ligand 1 (PD-L1) expression on monocytes and neutrophils in whole blood using a custom three-color immunopheno-typing panel. PD-L1, also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1), is a protein that, in humans, is encoded by the CD274 gene. PD-L1 has been thought to play a major role in suppressing the immune system during particular events such as autoimmunity. In mouse models, activated monocytes have been shown to greatly up-regulate PD-L1. PD-L1 has been shown to act as a positive co-stimulatory molecule in intracellular infection. During FY2016, AML analyzed PD-L1 expression in 323 patients.
- In support to Dr. Frank Maldarelli, NIAID, the AML Clinical Flow Cytometry Group re-analyzed flow cytometry data from patients enrolled in the Potential for Interferon Therapy in HIV-infected and ARV-suppressed Participants (PITHA) clinical trial. This trial examined the effect of pegylated interferon alpha 2b intensification on HIV-1 residual viremia in individuals suppressed on antiviral therapy. AML provided mean channel fluorescence data for HLA-DR/CD38, CD25, and CD27/CD45RO on CD4+ and CD8+ T lymphocyte subsets. During FY2016, AML re-analyzed mean channel fluorescence data in 90 patients.

- In support to Dr. Damilola Philips, NIAID, the AML Clinical Flow Cytometry Group re-analyzed flow cytometry data from individuals participating in the RPHI, RASFA, and RASFB clinical trials. During FY2016, AML provided immunophenotype results on CD4/CD38/CD45RO and CD8/CD38/CD45RO lymphocyte subsets for 376 patients.
- **Ebola Virus Disease Project**. In support of the NIAID-sponsored Ebola vaccine clinical trials that are currently being conducted in West Africa, a full-time staff member of AML provides laboratory support to the ongoing clinical trials in Liberia. Nadeeka Randunu, a research associate, is responsible for performing clinical assays, and trouble shooting and maintaining laboratory equipment at three clinical laboratories in Liberia. He is also responsible for training Liberian support personnel. Mr. Randunu currently rotates duties two months at a time in West Africa and one month in the U.S.
- **Biomarker Testing**. During FY2016, the AML Functional Immunology Group continued to perform ELISA tests and electrochemiluminescent multiplex assays to measure a wide range of biomarkers in support of multiple NIAID-sponsored clinical trials and research projects. Testing activity included:
  - In support to Dr. H. Clifford Lane, NIAID and the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) network, the laboratory successfully optimized a MesoScale-based assay for measuring IL-27 in human plasma.
  - In support to Dr. H. Clifford Lane, NIAID and the INSIGHT Network, the laboratory retrospectively measured seven biomarkers of inflammation in 8,296 acute plasma specimens collected from individuals who participated in the Strategic Timing of AntiRetroviral Treatment (START) clinical trial. Biomarker assays for d-dimer, IL-6, IL-27, CRP, SAA, ICAM-1, and VCAM-1 were completed on 4,308 baseline specimens and 3,988 month-8 specimens. The assays were completed in a very short timeframe (approximately 1 month) with the assistance of the Tecan robotics system. Biomarker results were provided to NIAID in four batches, with the final transmission of data occurring well ahead of the project deadline.
  - In support to Dr. Irini Sereti, NIAID, the laboratory measured IL-27 levels in 39 plasma specimens from the College Fund project.
  - In support to Drs. Irini Sereti and Virginia Sheikh, NIAID, AML measured levels of IL-7 and soluble-CD200 in 166 plasma specimens.
  - In support to Drs. Irini Sereti and Andrea Lisco, NIAID, AML measured a panel of 22 cytokines in 37 cell culture supernatants.
  - In support of Dr. Frank Maldarelli, NIAID, the laboratory measured a panel of six biomarkers in 140 plasma specimens obtained from HIV-infected individuals.
- **AML Research Flow Cytometry Group.** The AML Research Flow Cytometry Group provides flow cytometry support to DCR, NIAID (Drs. Anthony Fauci and H. Clifford Lane). During FY2016, this support included 26 sterile cell sorting procedures, 79 CSFE/cell proliferation assays, 277 cell-cycle analyses and six Robosep cell sorting procedures. In addition, the following flow cytometric determinations were performed: seven eleven-color, 32 nine-color, 652 eight-color, 91 seven-color, 36 six-color, 30 five-color, 396 four-color, 44 three-color, 593 two-color, and seven single-color. Specific efforts are listed below.
  - **Dr. Irini Sereti, NIAID**. The laboratory continued to perform complex seven and eight-color immuno-phenotypic analysis to investigate memory and naïve subsets of CD4 and CD8 cells, and T-reg cells in patient specimens collected from the IRIS clinical trial. Thirty patient specimens were analyzed during FY2016.
  - **Dr. Irini Sereti, NIAID**. The laboratory performed five cell sorting experiments to obtain ultrapure populations of CD4-positive and CD4-negative cells in support of a project to study the effects of tuberculosis/HIV co-infection on long-term HIV reservoirs in HIV-infected individuals.
  - **Dr. Colleen Hadigan, NIAID.** The laboratory continued to perform complex five-color immunophenotypic analyses of blood specimens in support of the Clinical Outcomes in Persons with HIV Acquired Early in Life (COPE) clinical research protocol. Six patient specimens were analyzed during FY2016.
  - **Dr. Hiromi Imamichi, NIAID**. The laboratory performed a variety of immunophenotypic determinations and cell sorting experiments in support of several HIV proviral-DNA sequencing projects.
  - **Dr. Tomozumi Imamichi, Leidos Biomedical Research, Inc.** The laboratory continued to perform a variety of cell cycle and cell surface marker assays to study the mechanism by which interleukin-27 inhibits HIV-1 replication in T cells, macrophages, and dendritic cells in vitro.
- **Biorepository Support to NIAID.** AML continued to serve as the central biospecimen repository for several domestic and international clinical trials conducted by the NIAID Influenza Research Collaboration (IRC) group and the NIAID INSIGHT group. Specific biorepository projects are identified below.
  - **IRC001B:** A Pilot Study for Collection of Anti-influenza A (H1N1) Immune Plasma. During the past year, AML received, inventoried, processed,

and stored approximately 300 serum samples per week from three community blood banks: Mississippi Valley Regional Blood Center, Davenport, Iowa, Memorial Blood Centers, Saint Paul, MN, and Gulf Coast Regional Blood Center, Houston, TX. The specimens were processed and screened for high levels of anti-influenza antibodies in order to be used for future influenza A (H1N1) immune plasma treatment studies or for use in the manufacture of high-titer H1N1 intravenous immunoglobulin. AML processed approximately 7,300 serum specimens during the 2015/2016 influenza season.

- **IRC003 and IRC004:** International, Multicenter Influenza Treatment Studies. During the past year, AML coordinated the receipt, inventory, and storage of approximately 8,950 IRC003/ IRC004 biospecimens (sera, oropharyngeal swabs, and nasopharyngeal swabs) collected from patients treated at 41 U.S. clinic sites and 15 international clinic sites. The laboratory also shipped 943 specimens to the Naval Health Research Center (NHRC), San Diego, CA for protocol-mandated virology testing. Staff collaborated with Social & Scientific Systems (SSS), Inc. to provide standardized packaging for IRC003 domestic sites shipping to the central biospecimen repository in order to streamline the process and ensure consistency of shipments during the past influenza season. Staff collaborated with the SSS and the IRC study team to ensure timely shipment receipt and inventory/data reconciliation/analyses for the IRC003 study close-out in 2016 and IRC004 Data and Safety Monitoring Board review in July 2016. Staff collaborated with the Clinical Monitoring Research Program Project Manager to review, outline, and resolve data queries within the weekly IRC003/IRC004 specimen tracking reports and virology test samples at the NHRC; including the identification of potential specimen collection protocol deviations that CTM will review and document with sites during study close-out visits for IRC003. Lab staff represented the NIAID IRC Repository on the IRC Study Team, providing logistical/repository support on the IRC Combination/Operational and IRC003 Domestic Team/Site teleconferences.
- **IRC005:** A Randomized Double-Blind, Phase 3 Study Comparing the Efficacy and Safety of High-Titer versus Low-Titer Anti-Influenza Immune Plasma for the Treatment of Severe Influenza A. The laboratory received, cataloged, and stored approximately 2,034 specimens (sera and oropharyngeal swabs) from patients treated at multiple clinic sites in the U.S. The laboratory processed and forwarded clinical specimens to the Virus Isolation and Serology Laboratory for anti-influenza antibody testing, and forwarded specimens to the Laboratory of Molecular Cell Biology for influenza virus detection.
- **INSIGHT FLU002 and FLU003:** International Observational Studies of Patients with Influenza. During the past year, AML coordinated the receipt, inventory, and storage of approximately 72,897 FLU002 and FLU003 biospecimens (sera, plasma, oropharyngeal swabs, nasopharyngeal swabs, and lower respiratory tract specimens) collected from patients in 18 countries. AML also coordinated the shipment of 96 INSIGHT FLU specimens to study investigators in support of various influenza research projects. AML continued to represent Leidos Biomed on the INSIGHT Laboratory Procedures Group and INSIGHT FLU Protocol Teams; reviewing clinical protocols, case report forms, lab manuals, and proposed use of specimens.

- **INSIGHT FLU006:** Anti-Influenza Hyperimmune Intravenous Immunoglobulin Clinical Outcome Study. AML served as the central biospecimen repository for a new, international randomized double-blind placebo-controlled trial of anti-influenza hyperimmune intravenous immunoglobulin (IVIG) in individuals hospitalized with influenza A or B, to determine whether, when added to standard of care treatment, IVIG helps reduce the severity and duration of flu symptoms. During the past year, AML coordinated the receipt, inventory and storage of approximately 1,512 FLU006 biospecimens and coordinated the shipment of 79 INSIGHT FLU006 specimens to study investigators in support of various influenza research projects. AML continued to represent Leidos Biomed on the INSIGHT FLU006 Protocol Team.
- **INSIGHT PREPARE Study:** A Multicenter Study of the Immunogenicity of Recombinant Vesicular Stomatitis Vaccine for Ebola-Zaire (rVSVΔG-ZEBOV-GP) for Pre-Exposure Prophylaxis (PREP) In Individuals at Potential Occupational Risk for Ebola Virus Exposure. Staff assisted with preparatory work necessary for the laboratory to serve as the central biospecimen repository for the PREPARE study; including BSI study set-up, sample ID reservation, and networking teleconferences between Leidos Biomed, NIH, and INSIGHT SDMC.
- **Yellow Task #14-099**. In support to NIAID DCR, AML continued to serve as the technical project manager (TPM) for a subcontract with Advanced BioMedical Laboratories, LLC (ABML), for the storage of approximately 330,000 legacy biospecimens collected from various INSIGHT studies, such as ESPRIT, SMART, STALWART, and CPCRA IL-2 clinical trials. The TPM reviewed and approved monthly invoice statements received from ABML. The TPM assisted Leidos Research Contracts with exercising Option Year One of the contract, and the TPM assisted in modifying the contract to increase

the number of -80 storage freezers from 18 to 20. In September 2015, the TPM assisted with the procurement and shipment of two new -80 freezers to ABML in order to replace existing, aging freezers.

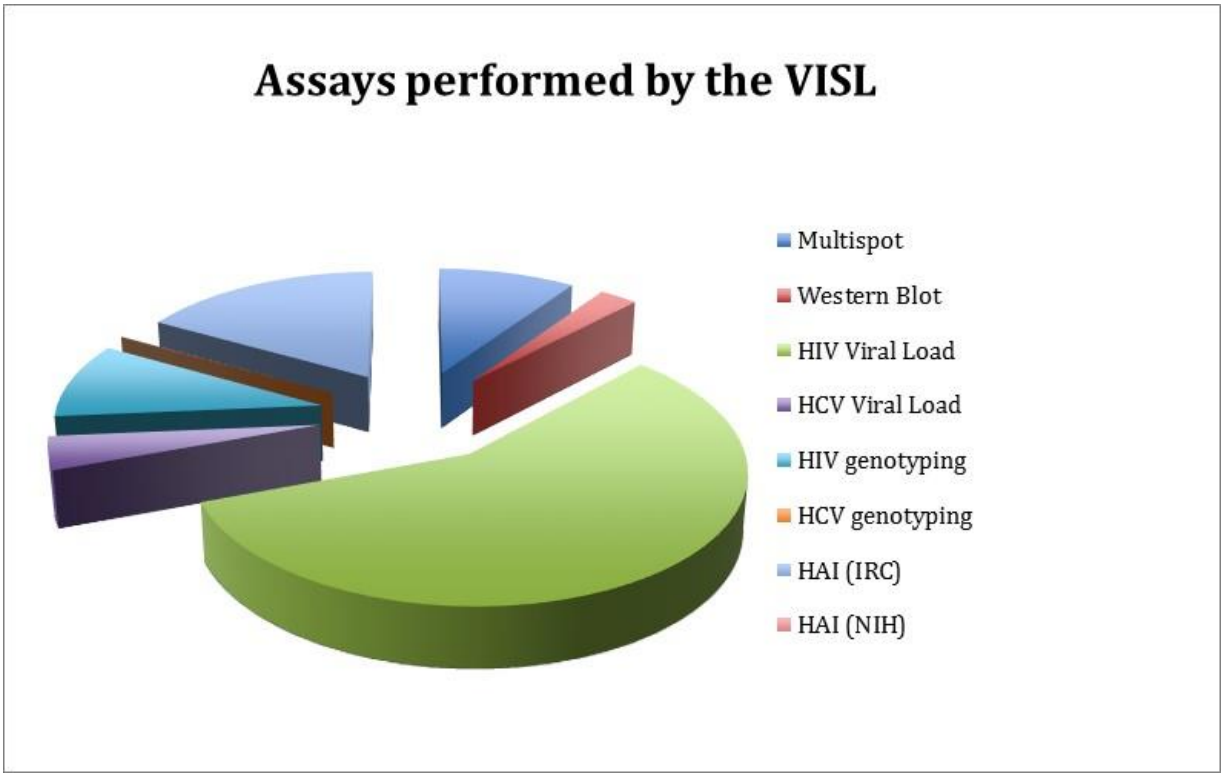
Virus Isolation and Serology Laboratory

The Virus Isolation and Serology Laboratory (VISL) provides services to the Laboratories of Immunoregulation, Clinical Investigations, Host Defenses, and the Division of Intramural Research within NIAID.

The primary mission of the VISL is to perform sequential serologic, virus detection and quantification, and genotyping studies. The results of these studies provide information for the further development of research and support the ongoing drug efficacy and epidemiology studies of the NIAID/Laboratory of Immunoregulation.

SIGNIFICANT ACHIEVEMENTS

- The first was the complete transition from Trugene HIV genotyping to Illumina next generation sequencing (NGS). After 17 years of using the Trugene platform, Siemens discontinued the assay, and we needed to find a new way to monitor HIV drug resistance in clinic patients. After experimenting with different lengths of HIV genome from 2.8 to 9 kb, we settled on 4.8 kb for coverage of key resistance-related regions. Due to the increased depth this more complex procedure provides, our accomplishment will enable us to provide NIH physicians with an abundance of information regarding drug resistance, virus biology, tropism, haplotype, and potential new candidates for antiretrovirals.
- Our second big accomplishment this year was the acquisition of the Cepheid GeneXpert instrument in order to evaluate the HIV-1 viral load assay for our customer, Dr. Clifford Lane. Although the instrument is available in this country for an assortment of analytes, it is not yet approved for HIV viral load. Dr. Lane would like to use the system for an NIAID project in Indonesia, and asked us to compare it to our gold standard here for HIV viral load– the Abbott m2000 system. Cepheid has never had anyone in the U.S. ask to borrow an instrument for evaluation, and the HIV kits are not sold here. However, after much negotiation, the company agreed to loan us a 16 module unit, and arranged for us to purchase 25 kits from Europe where the assay is currently in use. In conjunction with NIH and AML, we were able to obtain more than 200 samples to run in parallel on the GeneXpert and Abbott instruments, and completed our evaluation in less than two months. Not only was Dr. Lane pleased with our results, he encouraged us to purchase a unit for our lab in order to make quick turnaround HIV viral loads available to the 8th floor clinic.



Multispot	454
Western Blot	127
HIV Viral Load	2731
HCV Viral Load	200
HIV Genotyping	457
HCV Genotyping	2
HAI (IRC)	800
HAI (NIH)	0

Laboratory of Molecular Cell Biology

Testing for and quantitation of influenza viral RNA

Laboratory of Molecular Cell Biology as one of the two central laboratories for testing influenza samples for an international epidemiological study has tested 1882 nasal, oral, and endotracheal swab samples for the presence of pandemic influenza, seasonal H1, H3, and influenza B viral RNAs. Samples that were indeterminants were grown in culture and retested for the influenza A and B viral RNAs. During this review period, this laboratory also tested samples from IRC005 protocol. For this study a total of 155 nasal swab samples were tested for the presence of pandemic influenza, AH1, AH3, and influenza B viral RNA and then the virus present was quantitated by real-time PCR. For an IVIG study, 89 samples were subtyped into flu AH1 and AH3 and influenza viral RNA in these samples were quantitated by using an in-house developed method.

Detection and quantitation of gene-modified cells in patients' samples

Patient samples from three different clinical trials were monitored for the presence of gene-modified cells by quantitative PCR. Data show that some patients maintain a significant number of gene modified CD4+ and CD8+ T cells in their blood for more than 20 years after the infusion of those cells.

Detection of HIV DNA in patients' samples

Samples from 10 patients were tested for HIV gag, envelope, and long terminal repeat sequences.

Detection and quantitation of HIV DNA and RNA from human brain samples

An NIAID initiated project required a method to detect and quantify HIV DNA and RNA levels in brain samples. Human brain samples from parietal, temporal, and frontal cortex were obtained from control and HIV positive individuals. Different DNA and RNA isolation methods were tested and the methods that yielded the highest quality and quantity of plasma RNA and cell associated DNA and RNA were selected for the study. Estimation of HIV RNA and DNA in these samples showed the presence of detectable HIV DNA and RNA in brains of HIV positive patients

Quantitation of Simian Immunodeficiency viral RNA

Methods were optimized to quantitate cell associated SIV RNA and DNA as well as SIV RNA in plasma with a detection limit of five copies per ml. Using this in-house method SIV RNA was quantitated from 75 macaque plasma samples.

The sodium/iodide symporter (NIS) gene detection

We have established an assay to detect the sodium/iodide symporter (NIS) reporter gene in macaque cells for a study on in vivo imaging of adoptively transferred hematopoietic cells in macaques. NIS levels in 43 samples were quantitated in macaque and human cells for an NIAID investigator to optimize the conditions for gene transduction methods.

SNP samples

Twenty samples from patients were genotyped for three different loci of the ATP-binding cassette, sub-family B1 (ABCB1) gene.

Inhibition of HIV replication by Guanabenz

Guanabenz (GBZ) and Sephin1 (SPH) are small synthetic compounds that have been shown to prolong the phosphorylation of the translation initiation factor, eIF2α, during endoplasmic reticulum stress. Recent results from Laboratory of Molecular Cell Biology (LMCB) have shown that GBZ inhibits vesicular stomatitis virus replication by inducing eIF2α phosphorylation, induction of stress-response genes, and formation of stress granules. The role of ER-stress-mediated pathways in modulating HIV-1 infection is not well understood. In this study the effect of GBZ and SPH on the HIV-1 replication was investigated. Experimental results show that GBZ and SPH, small synthetic compounds that have been shown to prolong the phosphorylation of eIF2α during endoplasmic reticulum stress, inhibit HIV-1 replication.

Laboratory of Human Retrovirology and Immunoinformatics

The Laboratory of Human Retrovirology and Immunoinformatics (LHRI) is composed of three groups: the basic research group, the clinical research group, and the bioinformatics group. The basic research group has continued to evaluate potential anti-HIV activity in novel reagents and investigated

the mechanism of the antiviral effect. The clinical research group has provided services to investigators at NIAID to support ongoing clinical trials and projects, and the bioinformatics group have conducted bioinformatics analysis to support basic and clinical research. LHRI has performed basic and clinical research with the goal of gaining a better understanding of the immunopathological effects of infectious diseases on the host and how these effects are influenced by the genetics of the infectious agent and/or the host.

Basic Research Section

The primary goal of the research in the LHRI basic research group is to develop potent anti-viral therapies using cytokines or immune regulatory reagents for HIV- or HIV-related virus infected patients who have virologically or immunologically failed in clinical therapy at NIAID. In the course of studies, the group has found a broad antiviral effect in IL-27 (J.M. Fakruddin et al., Blood 2006, T. Imamichi et al., AIDS 2007, A.C. Frank et al., *JICR* 2011, L. Dai et al., *J Expt Med* 2013), discovered novel micro-RNAs possessing potent antiviral activity (S. Swaminathan et al., *BBRC* 2013) and disclosed a novel innate immune activation pathway that has shed a new light on how the immune system functions.

Identification of Y-box binding protein 1 (YB-1) as a host factor that regulates HIV infection in T cells: The LHRI basic research group has previously identified interleukin (IL)-27 as an antiviral cytokine that inhibits HIV-1 in T cells, macrophages, and dendritic cells in vitro. In previous studies, the group reported the molecular mechanism of the anti-HIV effect in macrophages, however the mechanism of the anti-HIV effect remained unclear. During the course of study to elucidate a mechanism underlying the antiviral effect in T cells, LHRI has found that YB-1, a transcriptional factor, plays a key role in the antiviral effect. IL-27 down-regulates the phosphorylation of YB-1 and subsequently the IL-27–treated T cells appear to be resistant to HIV and HSV-1 infection without impacting the cytokine production profile. This finding was presented in the Cold Spring Harbor Laboratory meeting for retrovirus (D. Poudyal et al., May, 2016) and currently the manuscript is in preparation.

Investigation into the mechanism by which IL-27 inhibits HIV in Dendritic cells (DC): The LHRI basic research group has previously reported that IL-27 inhibits HIV infection in DC (Q. Chen, et al., *PlosOne*, 2013) and continued investigation into the mechanism of the anti-HIV effect. During the course of the study, LHRI discovered that IL-27 induces HIV-resisting DC from monocytes during differentiation. Affymetrix microarray and bioinformatics analyses revealed that Anlyrin Repeat Domain 22 (ANKRD22) is induced in DC. Knock down study using small interfering RNA against ANKRD22 demonstrated that the protein may play a pivotal role in the antiviral effect in only DC. Further molecular study is currently under way.

Evaluation of anti-HIV effect in novel micro RNA (miR): The LHRI basic research group previously discovered six novel miR (hsa-miR-6852, -7702, -7703, -7704, -7705, and -7706) in IL-27 treated macrophages (S. Swaminathan, et al., *BBRC* 2013) and reported that those miRs potentially possess antiviral properties; however, precise analysis of the anti-HIV activity has not been carried out. In this period, the LHRI basic research group evaluated biological function in each miR and found that has-miR7702 possesses antiviral activity against HIV-1 and has-miR6852 induces cell cycle arrest at Gap 2 (G2) in several proliferating cells. Currently manuscripts describing each function are in preparation.

Characterization of the mechanism of DNA-mediated IFN-λ1 induction: The LHRI basic research group previously reported for the first time that interferon lambda 1 (IFN-λ1), a potent anti-HIV cytokine, is induced in macrophages, dendritic cells, and monocytes as an innate-immune response against cytosolic DNA (e.g., DNA from an infected DNA virus). LHRI discovered that the induction is mediated via Ku70, a protein related to DNA repair (X. Zhang, et al., *J Immunol*, 2011, H. Sui, et al., *Nuc Acid Res* 2014). Recently LHRI found that K70 forms a complex with stimulator of interferon genes (STING) and TANK binding kinase 1 (TBK1), which plays a key role to induce down-stream signal for the IFN-λ1 induction. In this period, LHRI has further investigated the molecular mechanism of the Ku70-STING-TBK1 complex mediated IFN-λ1 activation, and then found that the cytosolic DNA initiates the translocation of Ku70 (it endogenously locates in the nuclear) from the nuclear to the cytosol, and then Ku70 forms a complex with STING/TBK1. The complex induces activation of IFN-regulatory factors (IRF)-1 and 7 followed by IFN-λ1 gene activation (Figure 1). The finding has been presented in Keystone Symposia for nucleic acid sensing pathway (H. Sui, et al., 2016), and a manuscript describing the findings has been submitted.

Examination of the relationship between plasma interleukin (IL)-15 levels in a well-characterized cohort of HIV-1 infected patients: LHRI basic research group previously reported that IL-15 is able to suppress HIV infection in a certain T-cell types (R. Oguariri, et al., *J Bio Chem*, 2013). To elucidate in vivo relevance, in collaboration with AML and the NIAID Biostatistics Research Branch, LHRI has examined the relationship between plasma IL-15 levels and other biomarkers in a well-characterized cohort of HIV-1–infected patients. LHRI found that IL-15 levels were significantly elevated in HIV-infected patients with viral loads >100,000 copies/ml (3.02 ± 1.53 pg/ml) compared to both uninfected controls (1.70 ± 0.38 pg/ml, $p<0.001$) or patients with a viral load <50 copies/ml (1.58 ± 0.39 pg/ml, $p<0.001$)

(Figure 2) There was a significant correlation between HIV-1 viremia and IL-15 levels (Spearman r=0.54, p<0.001) and between CD4+ T-cell counts and IL-15 levels (Spearman r=-0.56, p<0.001). These data support a potential role for IL-15 in the pathogenesis of HIV-associated immune activation. A manuscript describing the findings has been submitted (S. Swaminathan et al.).

Fig. 1 Novel mechanism of the DNA-mediated IFN-λ1

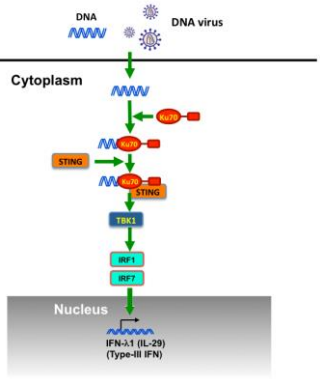
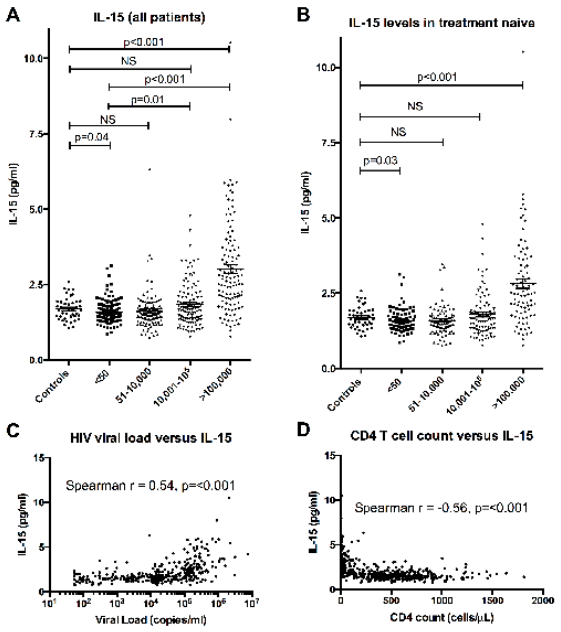


Figure 2. Correlations among IL-15, HIV virus load and CD4 counts



Clinical Research Section

The LHRI clinical research group serves as an Affymetrix Core Laboratory that provides assistance in all or part of the design, assay performance and analysis of gene array experiments. LHRI offers services for most gene arrays that Affymetrix produces. The arrays requested for processing this last fiscal year were the Human Gene 2.0 ST Array and the miRNA 4.0 Array. Request for RNA extraction may be included as part of the processing for Human Gene 2.0 ST Arrays. LHRI has the capability of providing various methods of RNA extraction depending upon the type and amount of starting material, RNA product required, and the downstream application. LHRI also offers services to assist with next generation sequencing projects. We provide DNA extraction procedures, which includes analysis of the quality and quantity of the nucleic acid, and shipping of the DNA to the agreed upon vendor for sequencing or returned to the investigator.

Samples Processed by LHRI:

- In support of Dr. Patrizia Farci, NIAID/LID/HPS, HIV studies, 254 samples were hybridized to the miRNA 4.0 Arrays and subsequently stained, washed, and scanned. The data was sent to the client for analysis.
- In support of our CSP collaborators, 56 samples were hybridized to the Human Gene 2.0 ST Arrays and subsequently stained, washed, and scanned. The data was analyzed by our bioinformatics group and reported to the collaborator.
- In support of Joe Kovacs, NIAID/CCMD HIV/AIDS Program, 180 samples were hybridized to the Human Gene 2.0 ST Arrays and subsequently stained, washed, and scanned.
- In support of Dr. Irini Sereti, NIAID, DNA was isolated from 8 clinical samples for subsequent NGS.
- In support of Dr. Joseph Kovacs, NIAID/CCMD HIV/AIDS Program, DNA was isolated from 60 clinical samples for subsequent NGS.

Bioinformatics Section

The bioinformatics group serves as a core data analysis laboratory for customers in NIAID upon request. Data analysis services include microarray analysis, analysis and bioinformatics pipeline development for NGS, and big data experiments as well as maintenance of the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/), a bioinformatics tool suite.

Bioinformatics pipeline for HIV genotyping and drug resistance report generation: In support of Dr. Robin Dewar with the VISL, the LHRI bioinformatics group developed a bioinformatics pipeline for HIV genotyping from NGS data obtained from HIV positive patient plasma samples. Additionally, scripts/programs were developed to obtain drug resistance information from the Stanford HIV program for each genotype and to format the information into a report format familiar to the NIH clinicians.

Full length HIV quasi-species characterization: The LHRI bioinformatics group developed an algorithm to deconvolute HIV quasi-species from PacBio sequencing data. Both quasi-species separation and the PacBio error profile are challenging for analysis, especially for full-length HIV sequences, given the limited abundance of the higher quality CCS reads. LHRI is using the algorithm to support Dr. Robin Dewar with the VISL and collaborate with Dr. Avindra Nath with the National Institute of Neurological Disorders and Stroke.

Ebola vaccine microarray study: The LHRI bioinformatics group determined gene expression patterns associated with a phase 1, double blinded, dose escalation trial of an rVSV-based vaccine candidate expressing the glycoprotein of a Zaire strain of Ebola virus (ZEBOV) in collaboration with Dr. Richard Davey (NIAID) and Dr. John Beigel (Leidos Biomedical, Inc.).

TCR repertoire sequencing and bioinformatics: A targeted TCR sequencing (TCRSeq) assay was developed and validated in house using a dog tag method for identifying PCR replicates. A bioinformatics analysis pipeline was developed and ideal PCR cycle # for low cell count samples was studied based on controls. Several patient samples were sequenced and processed with the pipeline in support of Dr. Irini Sereti (NIAID). Currently the protocol for TCRseq is in progress.

Pneumocystis microarray and sequencing studies: LHRI contributed to several Pneumocystis-related studies in support of Dr. Joseph Kovacs (NIH Clinical Center) including:

- Characterization of chemokine and chemokine receptor expression during Pneumocystis infection in healthy and immunodeficient mice
- Caspofungin effects on gene expression of immune response in Pneumocystis infected mouse
- Pnuemocystis major surface glycoprotein (MSG) Effects on Gene Expression in Mouse Dentritic Cells
- Effects of MSG on Rat Alveolar Type I Epithelial Cells
- Generation of three high-quality Pneumocystis genome assemblies, including the first P. murina genome assembly and new assemblies for P. carinii and P. jirovecii.

D-dimer GWAS study: The LHRI bioinformatics group conducted GWAS analysis for samples from ESPRIT and SMART cohorts to test if genomic correlates with D-dimer levels could be determined. The results were presented at the 2016 INSIGHT meeting.

Whole exome and whole genome analysis: LHRI conducted whole exome and whole genome sequence analysis for samples from patients with immuno-deficiency in support of Dr. Cliff Lane and Dr. Mary Wright. LHRI has found that a total of 28 single nucleotide polymorphism (SNP) on 19 immune response/related genes in the patient. One of the SNP was confirmed as a novel SNP by Sanger DNA sequence conducted by the LHRI basic research section, and the SNP has been submitted to the SNP data base.

Viral integration discovery: LHRI established a bioinformatics pipeline to identify and compare viral integration sites from cells that were transduced with the rkat4SVGF3e− retroviral vector containing a CD4/CD3-zeta chimeric-receptor coding sequence, to create HIV-specific cytotoxic T cells in support of Dr. Ven Natarajan with the LMCB.

Neutrophil Monitoring Laboratory

The Neutrophil Monitoring Laboratory (NML) performs studies of phagocytic cell function on cells isolated from patients with recurrent bacterial, mycobacterial, and fungal infections (chronic granulomatous disease, Job's syndrome, leukocyte adhesion deficiency, IFN-γ receptor deficiency) as well as patients with abnormal inflammatory responses (PAPA syndrome). During the past year, the NML received 1,318 samples from patients and normal volunteers. Among these are 260 samples that were received by overnight express mail. Analysis of samples by overnight express prevents the costly expenses incurred for patient travel and housing. The NML performed 8,269 assays of neutrophil function (O_2 generation, staphylocidal activity, adherence to plastic/coated surfaces, chemotaxis, degranulation, and surface antigen expression by flow cytometric analysis). The NML work scope also includes analysis of cytokine production (up to 10 cytokines) by stimulated peripheral blood mononuclear cells (PBMC) using a multi-array cytokine analysis. An extensive panel of cellular stimuli, including many toll-like receptor ligands, is used to determine the integrity of specific ligand-receptor cell signaling that may be associated with specific immune dysfunction. Over the past year, 355 assays of cytokine production by cultured PBMC were performed. In the performance of the above work, the NML has determined 26,884 analytes (cytokines, lactoferrin, gelatinase, defensins, and other immunoregulators) by multiplex array and ELISA.

Characterization of patients with chronic granulomatous disease

Diagnosis of chronic granulomatous disease

Chronic granulomatous disease (CGD) is a rare genetic immunodeficiency that is caused by mutations in *CYBA*, *CYBB*, *NCF1*, *NCF2*, and *NCF4*, encoding for $p22^{phox}$, $gp91^{phox}$, $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ of the NADPH oxidase enzyme complex (NOX2). CGD is characterized by a failure of phagocytes (neutrophils, monocytes, macrophages, and eosinophils) to generate superoxide and other

related reactive oxygen species (ROS), leading to recurrent infections, granulomatous complications, and premature death. To diagnose CGD, the NML offers several assays that assess ROS production by patient neutrophils. Once a diagnosis of CGD is established, the NML performs immunoblotting of neutrophil extracts to characterize the specific protein deficiency in patients with CGD. Using only one vial of frozen neutrophils ($5x10^6$ cells), the NML can perform four separate Western blots that are developed with antibodies provided by the Laboratory of Host Defenses. In the past year, the NML analyzed polymorphonuclear Leukocytes (PMNs) from 43 patients and normal volunteers and identified the following defects:

- $p22^{phox}$ defect (immunoblot negative) – 1 individual
- $p22^{phox}$ defect (immunoblot positive) – 1 individual
- $p47^{phox}$ defect (immunoblot negative) – 9 individuals
- $p67^{phox}$ defect (immunoblot negative – 4 individuals
- $gp91^{phox}$ defect (immunoblot negative) – 12 individuals
- $gp91^{phox}$ defect (immunoblot positive) – 3 individuals

Based on the identity of the defective protein by immunoblotting, the NML forwarded DNA and RNA samples to the Laboratory of Molecular Technology, Leidos Biomedical Research, Inc. for genomic and cDNA sequence analysis. In the past year, the following mutations have been determined.

- $gp91^{phox}$ (X-linked CGD) – 31 patients, carriers, or kindred
- $p22^{phox}$ (autosomal CGD) – 5 patients
- $p67^{phox}$ (autosomal CGD) – 7 patients
- $p47^{phox}$ (autosomal CGD) – 1 patient
- $p40^{phox}$ (autosomal CGD) – 2 patients

Sequence analysis of patients with $p47^{phox}$ CGD

CGD is caused by defects in any one of five subunits of the phagocyte NADPH oxidase that include $p22^{phox}$ (less than 5 percent of CGD patients), $p47^{phox}$ (approximately 30 percent of CGD patients), $p67^{phox}$ (less than 5 percent of CGD patients), $gp91^{phox}$ (approximately 65 percent of CGD patients) and $p40^{phox}$ (one reported case). CGD neutrophils, monocytes, macrophages, and eosinophils fail to generate sufficient ROS, leading to recurrent infections, granulomatous complications, and premature death. In general, identification of the specific genetic defect in $p22^{phox}$, $p67^{phox}$, $gp91^{phox}$, and $p40^{phox}$ can be easily obtained by traditional Sanger sequencing. However, identification of the specific genetic defect in patients with $p47^{phox}$ CGD (gene *NCF1*) is complicated by the presence of two highly homologous (greater than 98 percent) pseudogenes that are thought to have arisen through gene duplication. The wild-type NCF1 gene has a GTGT at the start of exon 2, while the pseudogenes (*NCF1B* and *NCF1C*) contain a GT deletion (ΔGT) (Figure 1).

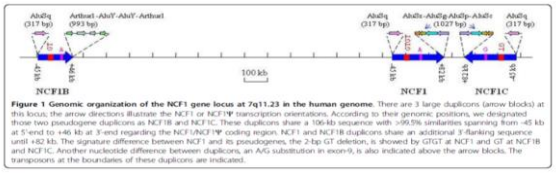


Figure 1 Genomic organization of the NCF1 gene locus at 7q11.23 in the human genome. There are 3 large duplicons (arrow blocks) at this locus; the arrow directions illustrate the NCF1 or NCF1Ψ transcription orientations. According to their genomic positions, we designated those two pseudogene duplicons as NCF1B and NCF1C. These duplicons share a 106-kb sequence with >99.5% similarities spanning from -45 kb at 5'-end to +46 kb at 3'-end regarding the NCF1/NCF1Ψ coding region. NCF1 and NCF1B duplicons share an additional 5'-flanking sequence until +82 kb. The signature difference between NCF1 and its pseudogenes, the 2-bp GT deletion, is showed by GTGT at NCF1 and GT at NCF1B and NCF1C. Another nucleotide difference between duplicons, an A/G substitution in exon-9, is also indicated above the arrow blocks. The transposons at the boundaries of these duplicons are indicated.

Brunson et al. BMC Genetics 2010 11:13 doi:10.1186/1471-2156-11-13.

Unequal crossover between the wild-type *NCF1* gene and these highly homologous pseudogenes is thought to account for the majority of mutations in $p47^{phox}$ deficient CGD by inserting the pseudogene-derived ΔGT into *NCF1*. Because of the high degree of homology between the wild-type gene and pseudogenes, standard Sanger sequencing has proven to be inadequate to assign a specific genetic mutation in these patients. We have developed a protocol using droplet digital PCR which can differentiate those patients with the ΔGT mutation from patients with other mutations in *NCF1* (Figure 2). Using this analysis, we can accurately segregate the populations into those CGD patients lacking the GTGT (86 percent of all $p47^{phox}$ CGD patients). The dotted lines represent the theoretical predicted values for one copy of GTGT/six total copies of *NCF1* and its pseudogenes (=0.17), two copies of GTGT/six total copies (=0.33), etc. Moreover, this assay can accurately determine the carrier status of other kindred within the $p47^{phox}$-deficient CGD families.

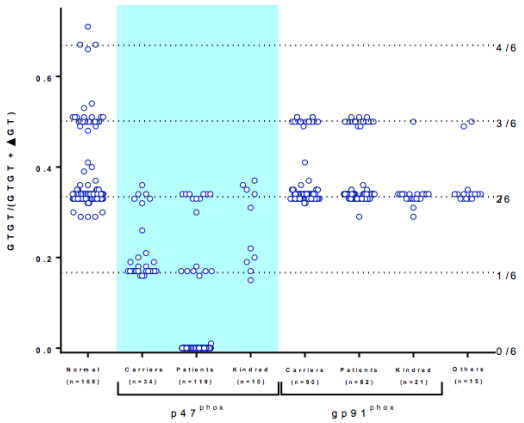


Figure 2. Droplet digital PCR determination of the number of copies of GTGT vs. ΔGT in normal subjects, obligate $p47^{phox}$ CGD carriers, $p47^{phox}$ CGD patients, $p47^{phox}$ kindred, $gp91^{phox}$ carriers, $gp91^{phox}$ CGD patients, $gp91^{phox}$ kindred, and others. The dotted lines represent the theoretical lines indicating 1 GTGT of 6 total copies (=0.17), 2 of 6 (=0.33), 3 of 6 (=0.5), and 4 of 6 (=0.67).

It is interesting to note that among the normal subjects tested, a significant number exhibited more than the expected two copies of GTGT with 16 individuals with three copies (13.7 percent of subjects tested) and 4 individuals with four copies (2.4 percent of subjects tested). These data suggest that while unequal crossing over can lead to insertion of the ΔGT into the *NCF1* gene, it can also lead to the unequal distribution of *NCF1* and its pseudogenes in daughter cells.

In the past year, we have analyzed an additional 267 DNA samples from our CGD cohort including many of our patients with X-linked CGD, their X-linked carrier mothers, and other kindred. These data demonstrate the specificity of the assay since none of the X-linked CGD cohort exhibited reduced GTGT/GTGT+ ΔGT) ratios. Moreover, in17 of 90 X-linked CGD carriers (18.9 percent) and 16 of 82 X-linked CGD patients (19.5 percent), there were of three copies of GTGT. These data resemble the findings observed in our normal population, albeit the percent is somewhat increased. This difference can be attributed to skewing of the population because multiple members of the same family are represented in the X-linked groups.

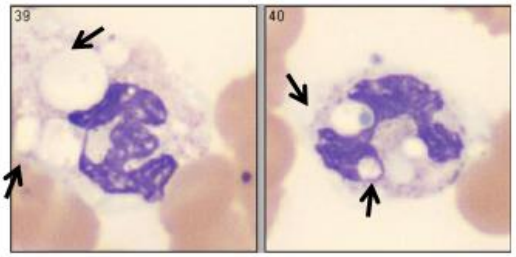


Figure 3. Whole Blood Smear Stained with Diff-Quik.
The two light micrographs represent neutrophils from a blood smear stained with Diff-Quik from patient with heterozygous RAC2 mutation; black arrows point to large cytoplasmic vacuoles.

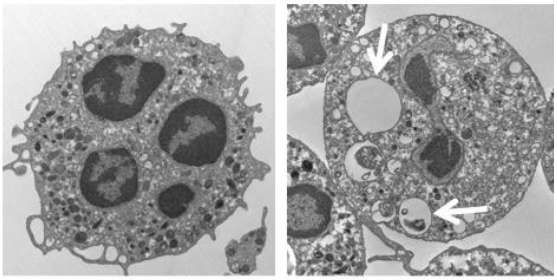


Figure 4. Transmission Electron Microscopy of Neutrophil
The image on the left illustrates the ultrastructural details of neutrophil from a normal volunteer; the image on the right illustrates the ultrastructural details of a neutrophil from the patient with heterozygous RAC2 mutation; white arrows point to large vacuoles in the patient neutrophil.

Characterization of Other Primary Immunodeficiencies

A. *Characterization of functional defects in a patient with a novel mutation in RAC2*

RAC2, a guanosine triphosphate-binding protein, plays a critical role in the activation of NADPH oxidases of human neutrophils (NOX2) in the reaction transferring an electron to O_2 to generate $O_2^{-\bullet}$. Mutations in RAC2 are characterized by impaired neutrophil functions, including the dysregulation of cell signaling, actin cytoskeletal organization, and cell migration, which result in recurrent infections and delayed wound healing. Thus far, patients have been reported with mutations in *RAC2* (dominant negative *RAC2*$^{+/-}$ D57N and recessive *RAC2*$^{-/-}$ W56X) that resulted in loss of function with impaired or reduced $O_2^{-\bullet}$ generation, F-actin assembly, chemotaxis, and primary granule exocytosis. Recently, a 38-year-old female with progressive severe lung disease presented at the NIH for routine screening prior to bone marrow transplantation. Whole exome sequencing, confirmed by Sanger sequence, identified a heterozygous *de novo* mutation in *RAC2* c.184G>A; p.E62K. Morphological studies revealed large cytoplasmic vacuoles visible by both light (Figure 3) and electron microscopy (Figure 4).

Extracellular ROS production was evaluated using superoxide dismutase-inhibitable ferricytochrome c reduction. Neutrophils from the patient with the *RAC2* c.184G>A; p.E62K exhibited a gain-of-function mutation with increased rate and duration of fMLF-induced ROS production (Figure 5).

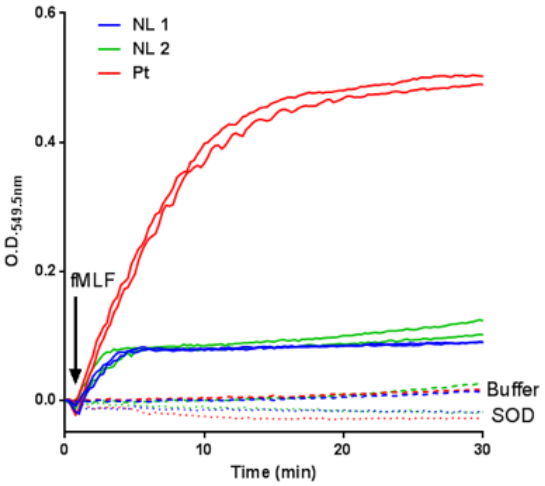


Figure 5 $O_2^{\cdot}$ Production by Cytochrome c Reduction
$O_2^{\cdot}$ production when stimulated with fMLF (solid lines) was monitored kinetically (every 15 seconds) for 30 minutes for normal subjects and patients. The arrow depicts the addition of fMLF. Dashed lines represent samples with addition of buffer alone; dotted lines, addition of superoxide dismutase (SOD) to verify specificity.

Assessment of actin cytoskeletal organization in patient neutrophils using the F-actin stain phalloidin staining and flow cytometry revealed increased F-actin content in patient neutrophils compared to neutrophils from a normal volunteer. Since RAC2 is known to be involved in cell migration, an assessment of cell migration was performed using EZ-TAXIScan instrumentation. Comparing the velocity of individually tracked neutrophils isolated from the normal volunteer and the patient, neutrophils from the patient cells exhibited markedly impaired random migration to buffer alone and directed migration (chemotaxis) to fMLF (Figure 6).

These data suggest that *RAC2* c.184G>A; p.E62K mutation results in a profound immunodeficiency associated with abnormalities in neutrophil morphology and cytoskeletal organization, ROS production, and both random and directed migration (chemotaxis). A bone marrow transplant in this patient has led to full engraftment and dramatic lung improvement.

Genetic analysis of other immunodeficiencies

In addition to providing sequence analysis for identification of the genetic defect in patients with CGD, the NML also provide sequence analysis for Crohn's disease, leukocyte adhesion deficiency, *WHIM* disease (warts, hypogammaglobulinemia, infection, and myelokathexis), and other neutrophil disorders. In the past year, the NML has isolated and forwarded 12 DNA samples to the Laboratory for Molecular Technology for genetic analysis associated with specific immune-deficiencies.

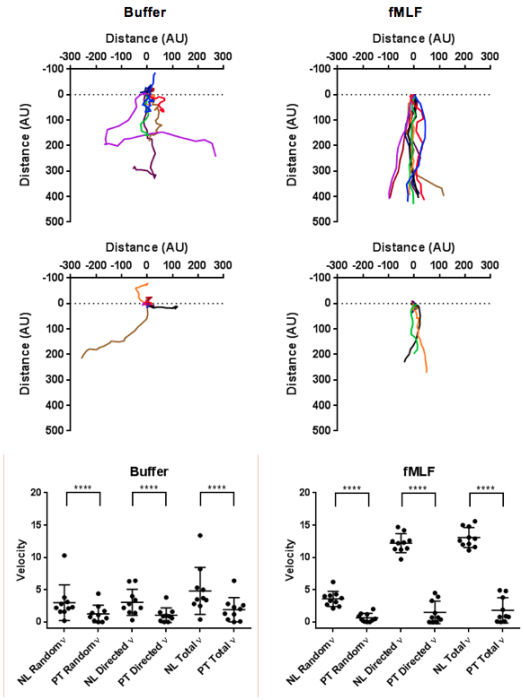


Figure 6. Heterozygous mutation in RAC2 results in impaired chemotaxis
Isolated PMNs ($5x10^3$in 1µl) were added to each "Cell" well of the EZ-TAXIScan instrument. One µl of buffer (left panels) or fMLF ($5x10^{-8}$M, right panels) was added to the "chemoattractant" well opposite the well containing the PMNs. EZ-TAXIScan software was set up to collect images of migrating PMNs in each of the six wells every 30 seconds for 1hour. Tracks of individual cells were subsequently analyzed using the MTrackJ tracking add-in software of ImageJ. The top panels illustrate tracks of individual neutrophils isolated from a normal volunteer (anchored at the origin for presentation). The middle panels illustrate tracks of individual neutrophils isolated from the patient. The bottom panels illustrate the summary data by plotting the random velocity vector (in X direction) of each individual cell, the directed velocity (in Y direction) of each individual cell, and the resultant total velocity of each individual cell from the normal subject and patient in the presence of buffer or fMLF.

- CXCR4 (WHIM disease) – 3 individuals
- ITGB2 (Leucocyte adhesion deficiency) – 1 individual
- WDR1 (Actin interacting protein 1) – 4 individuals
- IRAK4 (IL-1 receptor-associated kinase 4) – 4 individuals

Repository of Biological Samples for NIH investigators

The NML serves as a repository for patient biological samples collected under specific protocols by investigators at NIH. In the past year, 14,057 vials of plasma, serum, mononuclear cells, and neutrophils were stored in the Central Repository for future use. In addition, the NML has cultured an additional 306 EBV-transformed B cell lines, cryopreserving 3,335 vials. All cell lines were tested for mycoplasma

contamination to ensure that cell lines being forwarded to other investigators were contaminant-free. These cell lines have proven to be a valuable source of DNA and RNA to confirm genetic mutations in CGD. In the past year, the NML has established 26 primary fibroblast cultures from punch biopsies; and 293 vials of fibroblasts have been cryopreserved. In the past year, the NML prepared 183 shipments totaling 863 samples [DNA, RNA, EBV cell lines, plasma, and cells] for further analysis that were forwarded to other Leidos Biomedical Research, Inc. laboratories, to physician investigators at the NIH, and to outside testing facilities.

Expansion of the Neutrophil Monitoring Lab

Early in 2016, the NML expanded into an additional 465 square feet of laboratory space in the adjoining laboratory to relieve overcrowding in the current space and to accommodate an increasing work scope. An additional research associate I is being recruited to assist with the increased work scope.

Immunological Monitoring Laboratory

Over the last year, in support of the Laboratories of Clinical and Infectious Disease, the Laboratory of Host Defense, and the Laboratory of Infectious Disease, NIAID (APO/H 88-020), the Immunological Monitoring Laboratory (IML) has performed immune function studies on patients in a wide variety of protocols. The IML is continuing its involvement with a clinical trial for the treatment of Crohn's disease using the anti-IL-12 antibody, Ustekinumab. In collaboration with Drs. Lesia Dropulic and Jeff Cohen, the IML has completed the first human HSV2 vaccine protocol designed to prevent genital herpes disease. The vaccine is the ACAM-529, a live attenuated HSV viral strain backed by Sanofi Pasteur that has not yet been tested in humans. As the HSV529 protocol winds down the IML will enter into a pilot study directed by Dr. Lesia Dropulic and Ki Milligan evaluating safety, the anti-viral immune response, and estimating the disease frequency after human challenge with the wild-type recombinant respiratory syncytial virus A2 (RSV A2).

SIGNIFICANT ACHIEVEMENTS

- In the past 12 months, PBMCs, plasma and serum from 836 patient blood samples were isolated and cryopreserved from the patients on the protocols listed above. The IML isolated 3,994 vials of cells, 2,049 vials of plasma, 3,337 vials of serum, 1,083 vials of whole blood, and 179 vials of RNA and 79 frozen DNA lysates from these patients and volunteers.
- The IML is continuing its collaboration with the newly formed Novel Genetic Disorders of the Immune System section headed by Dr. Koneti Rao. This section has enlisted 21 new patients this year with novel genetic disorders that may or may not present autoimmune lympho-proliferative syndrome (ALPS) like symptoms, but do not have mutations in genes usually associated with ALPS (Fas or Caspase mutations). Since this is a new project we have begun the evaluation of several multiplex kit suppliers for our plasma cytokine testing, after using standard ELISA kits over the last twenty years for the ALPS study. This will allow us to evaluate a wide range of cytokines and chemokines now needed to investigate an expanded range of genetic disorders whose etiology is unknown.
- The IML, along with Drs. Warren Strober and Ivan Fuss have engaged in a new clinical protocol, looking at the mechanisms associated with Ustekinumab anti-IL-12 antibody treatment of Common Variable Immune Deficiency (CVID) patients who have associated GI involvement. We have successfully assayed the RNA levels of 14 cytokine and chemokine markers usually associated with the elevation of autoimmune symptoms associated with CVID from the first two patients before and after Ustekinumab treatment. Laminar propria cells harvested from these patients were stimulated in vitro with reagents that activate T cell and macrophage lineage cells. The results of the Quantigene assays have been sent to Dr. Fuss and are currently being evaluated.
- The IML recently concluded several Rhesus monkey vaccine protocols in collaboration with Drs. Jeff Cohen, Sarah Valencia, and Wei Bu in the Laboratory of Infectious Disease (LID); the LID 31#1 CMV vaccine study, and the LID 12 EBV vaccine study. After several early failed viral challenge attempts, the IML was able to quantitate viral DNA from urine and throat wash samples from the EBV and CMV challenged Rhesus monkeys by RT-PCR. Our data showed that the vaccines used to protect the monkeys from either the CMV and EBV challenge did not significantly change the viral gene copy levels in the vaccinated animals when compared to unvaccinated controls.
- The IML assayed multiple cytokines associated with patients having persistent hydro vacciniforme and other chronic EBV infections associated both T cell and B cell abnormalities some of which are associated with GATA2, Traf3, IRAK4, or unknown gene mutations. The patient's PBMCs were stimulated in vitro in the IML or Dr. Cohen's lab with stimulators associated with the activation of toll-like receptors TLR 1 through TLR 9. PBMCs from the patients with the various mutations did show differences in the degrees of responsiveness as determined by cytokine analysis to several of the

TLR stimuli, which may help explain the mechanism behind the persistence of the EBV infections in these patients.

- The IML has completed the HSV529 vaccine protocol. A protocol incorporating 60 volunteers that were vaccinated three times with the ACAM-529 live attenuated HSV strain over a six-month period with blood samples harvested and processed in the IML at 10 key intervals over a one-year period. At every interval, PBMCs and serum were isolated using a time sensitive protocol before being aliquoted, frozen, and stored in vapor phase liquid nitrogen. PBMC lysates were also produced and stored at every time point. All the PBMCs have been distributed to Dr. David Koelle at the University of Washington and the Center for Human Immunology & Inflammation (CHI), a trans-NIH initiative lab at the NIH. The Koelle lab, using Elispot assays was able to determine that the vaccine regimen was unable to induce a significant T-cell activation against the viral antigens in the vaccinated volunteers. The results were conclusive even though 13 percent of the PMBC samples sent to their laboratory were unable to be assessed due to bacterial contamination. The IML recently shipped to the CHI, PBMCs for FAX analysis, serum for cytokine analysis and serum neutralization assays, and PBMC lysates generated in the IML for the CHI to perform a transcriptosome analysis. All the samples have proven to be in excellent condition for all the various assays mentioned.
- In collaboration with Drs. Lesia Dropulic and Ki Milligan, the IML is close to completion of the protocol to evaluate the safety, the immune response, and estimating the disease frequency after human challenge in 28 normal individuals with the wild-type recombinant respiratory syncytial virus A2 (RSV A2). At seven intervals over a 56-day period after viral challenge, the IML has isolated PBMCs and serum from four cohorts between seven to ten individuals at each time point. The patient samples were processed in a time sensitive manner as prescribed by the protocol with PBMCs aliquoted at two concentrations, and serum aliquoted at three different volumes for the various assay to be performed. After the last sample has been processed, the vials will be distributed for assaying to the CHI, Johns Hopkins University and the NIAID, Laboratories of Infectious Disease of Drs. John Collins and Matt Manoli. Unfortunately, this protocol was ended before the anticipated number of challenge cohorts was performed due to the futility clause in this pilot protocol requiring a certain percentage of volunteers to shed virus over a pre-determined period of time. The investigators are considering another pilot study with an alternate RSV strain.

Division of Clinical Research Office of the Director Project/Program: Research and Laboratory Support (Radiochemistry)

ADRD provides staffing in Building 21 in Bethesda, embedded in the laboratory of Dr. Chaig Paik, head of the Radiopharmacy Section, Clinical Center. The Leidos Biomedical Research, Inc.'s senior scientist primarily provides support to Dr. Michele Di Mascio, chief, AIDS Imaging Research Section. Project support included the following:

- Continuing study: Longitudinal whole-body SPECT imaging of CD4+ by Tc-99m labeled F(ab′)2-CD4R1 following Initiation and Interruption of Antiretroviral Treatment in Rhesus Macaques (RM) and Immunogenicity Test.
  - Supporting SPECT imaging study with preparation of radiotracers of more than 20 doses of RM
  - Performing in vitro immunogenicity test: preparation of radiotracers and analysis plasma-cell binding assay by running High Performance Liquid Chromatography (HPLC) for more than 140 samples
- Whole body SPECT imaging of gp120 by Tc99m labeled anti-ENV (447-52D) in RM and in vitro tests (plasma-cell binding assay) in RM plasma
  - Performing in vitro plasma-cell binding assay for specific binding test by running HPLC for more than 35 samples and supporting autoradiography
  - Supporting SPECT imaging study of RM with preparation of radiotracer doses
- MicroPET Imaging and Autoradiography with Cu64/Zr89-MORAb-009 in Mesothelin+ A431/H9 tumor bearing mice
  - Performing autoradiography: Scanning and analyzing data of ex vivo tumor using Phospoimager (Typhoon FLA 7000; ImageQuant TL 8.1)
- MicroPET Imaging and Autoradiography with Zr89-B3 antibodies in Ley+ A431tumor bearing mice
  - Performing autoradiography: Scanning and analyzing data of ex vivo tumor using Phospoimager (Typhoon FLA 7000; ImageQuant TL 8.1)

Laboratory of Parasitic Diseases

The Clinical Support Laboratory (CSL) provides dedicated staffing to perform clinical sample processing for the Laboratory of Parasitic Diseases, including isolation of serum and plasma, cell isolations by density gradient separation, and immunomagnetic bead separation to isolate purified eosinophils. During FY2015, CSL received samples from 15 clinical protocols, with receipt of samples from 451 patient visits. CSL performed approximately 105 eosinophil isolations and prepared 5,820 aliquots of clinical materials for return to NIAID investigators.

Laboratory of Molecular Microbiology

CSL provides dedicated support to Dr. Malcolm Martin, Laboratory of Molecular Microbiology, for processing blood samples obtained from macaques and other nonhuman primates involved in simian immunodeficiency virus (SIV) vaccine studies. The laboratory received approximately 1,420 blood samples for separation of mononuclear cells and plasma, and generation of cell pellets for DNA extraction. Approximately 4,600 aliquots of mononuclear cells, 115 cell pellets, and 13,000 vials of plasma were produced for storage in the NCI at Frederick Central Repository or return to investigators as requested. The laboratory also prepared shipments of samples for return to Bethesda.

Additional ADRD Program Support

Data Management Group

The Data Management Group (DMG) was formed in 1990 to meet the needs of the Clinical Services Program's (CSP) scientific and administrative staff in support of NCI and NIAID initiatives. The DMG currently supports over 125 users and 300 equipment items, including network servers, workstations associated with scientific equipment, administrative and scientific staff workstations, and mobile IT devices. The DMG has been instrumental in the development of CSP program-specific database tracking systems and scientific computer programs. They constantly evaluate the computer needs of the CSP. Administrative and laboratory functions are analyzed to determine where procedures can be automated to save work hours. Workstations and networking equipment are monitored and upgraded to fit the growth of scientific data processing and storage. The group makes a consistent effort to provide the latest technology in networking and computer programming and ensures compliance with security requirements to protect data and patient confidentiality. The DMG consists of two sections: Programming Support and the Network Office.

SIGNIFICANT ACHIEVEMENTS

NCI

- AML 6 Color Flow Program - The DMG previously wrote a new program used by the AML Clinical Flow Cytometry Group for the extraction of six-color lymphosum monoclonal antibody testing result data used for clinical immunophenotyping. The program processes patient data in batches, eliminating many man-hours of manual data extraction and calculations. The program extracts the data and performs quality control (QC) checks for the generation of QC reports in addition to performing all the necessary flow analysis calculations required for the reporting of clinical data at NIH. During FY2016, the program was modified to incorporate the reporting of 6 color data for Dr. Robert Yarchoan.
- CSL Patient Tracking System – A new shipment module was added to the CSL Patient Tracking System for the shipment and reporting of samples for four National Institute of Environmental Health Sciences (NIEHS) protocols. This module replaced a manual process thus saving many man-hours by eliminating the entry of duplicate data into various locations. All sample data for the NIEHS protocols is now entered and tracked within the patient system. The modifications to the system required additional data fields to be added to the database and entry screen, which included the recording of DNA results that were not previously tracked within the system. The new DNA entry fields added to the system benefit all protocols requiring the processing of DNA samples. Additional program features included the establishment of data entry templates for each NIEHS protocol to ensure the accuracy and standardization of data entered by the laboratory. The new shipment module provides the laboratory with ease for the extraction of NIEHS sample data into external shipment files using a Biological Specimens Inventory (BSI) file specification provided by NIEHS. The external shipment files sent to NIEHS streamlines sample data reporting and importing of sample data into the BSI inventory system for NIEHS.

CSP Networking

The CSP DMG Network Office completed a major upgrade and consolidation of CSP network servers in support of NCI and NIAID programs. Several CSP servers were upgraded from Windows 2003 Operating System (OS) to Windows Server 2008 / 2012 (OS). Part of this initiative was also to reduce and consolidate the number of physical servers from a physical environment to a virtualized server platform for enhanced manageability. The

Microsoft Hyper-V environment was utilized to create a virtualized server environment for CSP development and production servers. The upgrade to the new Windows OS provides increased performance and maximum utilization of resources, which include application instances, data file security, space requirements, and server space allocations. The results of this initiative consisted of 10 new physical servers brought online and configured with the new Windows OS; 19 physical servers were removed; and 22 new virtual servers were established. This new structure allowed for a reduction in financial resources and an increase in server performance.

Laboratory of Cell-Mediated Immunity

In response to CSAS-17049, the Laboratory of Cell-Mediated Immunity (LCMI) performed three sets of three- and six-day proliferation assays to evaluate the proliferative response of cells from normal monkeys against the mitogen phytohemagglutinin, pokeweed, and a pool of allo-stimulator cells in a mixed lymphocyte culture, in the presence and absence of bone marrow stromal cells at multiple concentrations. A total of 174 tests were performed.

LCMI performed 1,289 proliferation, ELISPOT, and 51Cr-release assays at the request of Dr. Doug Kuhns, Leidos Biomedical Research, Inc., in support of CSAS-16922 and CSAS-16923.

LCMI performed 48 ELISPOT tests at the request of Dr. Ven Natarajan, Leidos Biomedical Research, Inc., in support of CSAS-17659.

Clinical Support Laboratory

In support of Dr. Michele Di Mascio, CSL performed analysis on approximately 275 blood samples, for a total of 1,150 tests, obtained from rhesus monkeys infected with SIV or from controls to evaluate lymphocyte subsets as part of a whole-animal imaging study. Plasma was also prepared and frozen from these samples. The samples were primarily received from a NIAID-supported animal facility in Poolesville, MD.

In support of Dr. Marta Catalfamo, CSL provided flow cytometry support to a nonhuman primate study of IL-15 and anti PD-L1 in SIV. The immunophenotyping panel evaluated changes in lymphocyte subsets, including memory and effector T-cell subsets, as well as activation and proliferation markers. A total of 36 nonhuman primate samples, for a total of 336 tests, were analyzed. Blood samples were also processed for isolation of PBMCs that were cryopreserved for future immune function analysis, and plasma was recovered and stored. In addition, the laboratory performed three assays on a total of 21 samples to evaluate if monkeys developed antibodies to IL-15.

CSL provided sample processing support to Dr. Michail Lionakis of the Fungal Pathogenesis Unit for Protocol 13-I-0187. Approximately 25 whole-blood samples were received for Ficoll separation and QIAzol treated aliquot preparation, with 240 aliquots stored in the NCI at Frederick Central Repository.

Support Provided by the Clinical Monitoring Research Program

Clinical Consulting and Support

The Clinical Monitoring Research Program (CMRP) Clinical Consulting and Support (CCS) group was established in the fall of 2004 to support the National Institute of Allergy and Infectious Diseases' (NIAID) special initiatives and projects. CMRP provides specialized project management, logistics, administrative, and programmatic support for various NIAID Division of Clinical Research (DCR) and Division of Intramural Research (DIR) initiatives, including establishment and maintenance of research subcontracts, financial management, building management, and overall administrative support. The CCS group has nine staff members.

During FY2016, CCS assisted in the ongoing management of approximately 35 active agreements of various types, coordinated conference arrangements, coordinated domestic shipments of clinical research material, printed and shipped plasma labels, coordinated the preparation and printing of two posters, completed 520 courier runs, and provided purchasing and property acquisitions support. Some activities supported by CCS staff have been reported under other project-specific sections.

Research Subcontracts Management

The ongoing management of active agreements of various types continued through the current reporting period. These agreements are for services such as clinical trials monitoring support to the CMRP Clinical Trials Monitoring (CTM) team for various NIAID labs conducting clinical trials, consulting services, and accreditation services. Assistance through the acquisition process was provided to the training group to establish an agreement with an organization to provide onsite customized training for a newly hired clinical research associate (CRA) within CTM. Additionally, two Pharmaceutical Product Development (PPD) task orders (TOs) for clinical monitoring of Protocol IRC 003 "A Randomized Double-Blind Phase 2 Study Comparing the Efficacy, Safety, and Tolerability of Combination Antivirals (Amantadine, Ribavirin, Oseltamivir) versus Oseltamivir for the Treatment of Influenza in Adults at Risk for Complications" and Protocol IRC 004 "A Randomized Double-

Blind Study Comparing Oseltamivir versus Placebo for the Treatment of Influenza in Low-Risk Adults" were transitioned from TO agreements associated with the prime Operations and Technical Support (OTS) contract to TO agreements associated with the new prime Indefinite Delivery/Indefinite Quantity (IDIQ) TO for respiratory diseases. In order to establish the new task order agreements, the acquisition process was initiated to solicit new technical and price proposals for review prior to award. Additionally, the acquisition process was also initiated during the current period to establish a TO with PPD to provide clinical monitoring support for a new protocol titled "Using Biomarkers to Predict TB treatment Duration" that will include study sites in China.

Modifications were also issued for two separate Knovex agreements. Modifications were issued under the Knovex task order agreement for leadership coaching services to the Associate Branch Chiefs within DCR, Program Planning and Analysis Branch (PPAB). The modifications aligned the statement of work with PPAB requirements, adjusted the timeline, and extended the TO period of performance and increased the budget for the FY2016 period. The second Knovex agreement, which is a stand-alone agreement, provides leadership coaching services for identified staff within DCR and was modified to extend the period of performance, increase the number of coaches, and increase the budget for the FY2016 period.

Throughout FY2016, the CCS group supported research subcontracts and agreements as follows:

- Managed research subcontracts with PPD, Inc., which include a basic ordering agreement (BOA) and multiple TOs for continued clinical monitoring efforts in support of ongoing international clinical trials. During the report period, there were seven active TOs, and five new TOs were added.
- Maintain a BOA with Bioanalytical Research Corporation (BARC) in the event repository type services in South Africa arise.
- Managed and closed out a consulting subcontract with Mr. Nicolaas Pool. Mr. Pool provided scientific and technical expertise support to the Phidisa Project.
- Managed research subcontracts with Ms. Ellen Cull to provide support for leadership and organizational development for the NIAID Office of Planning and Operations Support (OPOS).
- Managed and closed out a blanket order agreement with Progenitor Cell Therapy for the processing of peripheral HIV and blood stem cells for the "Immunologic and Virologic Response in HIV-Infected Progressors after Infusion of Lymphocytes from HIV-Infected 'Elite' Long-Term Non-Progressors" protocol. The services provided by Progenitor Cell Therapy were transferred to SriSai Biopharmaceutical Solutions, LLC during the reporting period.
- Managed a new agreement with SriSai Biopharmaceutical Solutions, LLC from pre-award through post-award. The agreement with SriSai Biopharmaceutical Solutions was executed to replace the Progenitor Cell Therapy agreement so that support for the processing of peripheral HIV and blood stem cells for the "Immunologic and Virologic Response in HIV-Infected Progressors after Infusion of Lymphocytes from HIV-Infected 'Elite' Long-Term Non-Progressors" protocol continued.
- Managed a BOA and one TO as well as one stand-alone agreement with Knovex, LLC in support of the DCR Leadership Development Initiative.
- Managed and closed out a research subcontract with Martin Michael, who provided support to the Barriers to Clinical Research initiative and was on standby during the reporting period to provide support to the implementation of a single Institutional Review Board (IRB) if initiated.
- Managed and renewed a research subcontract with the HIV Resistance Response Database Initiative (RDI) for modeling various antiretroviral therapy responses.
- Managed and renewed a research subcontract with Professional Education Services Group (PESG) to provide accreditation services for Intramural Clinical Management and Operations Branch (ICMOB) clinical staff.
- Managed and renewed a research subcontract with Delphine Yamadjako to provide clinical monitoring services for international clinical trials in West Africa.
- Managed and renewed a research subcontract with Khon Kaen University in Thailand to continue follow-up visits with subjects enrolled in an ongoing clinical study entitled "Mycrobacterial and Opportunistic Infections in HIV-Negative Thai Patients Associated with Autoantibodies to Interferon-y (NIAID Protocol 09-I-N060).'' Although the study supports the DIR, PPD is the clinical monitor for this study under an agreement in support of DCR.
- Managed a new agreement with Barnett International from the pre-award through post-award and then close-out stage, which was established to provide training on Clinical Trials Management to new Clinical Research Associates (CRAs).

- Managed and renewed research subcontracts and blanket order agreements with Digital Infuzion, Iron Mountain, Palladian, ALL-SHRED, and Fisher BioServices in support of the Office of Clinical Research Policy and Regulatory Operations (OCRPRO).
- Managed and renewed a research subcontract with Biologics Consulting Group, Inc. in support of DCR's research and development initiative for the recombinant human interleukin-15 (IL-15) study.
- Managed and renewed a research subcontract with Matthews Media Group to provide IRB meeting materials in support of OCRPRO.

Additionally, agreements were executed to support clinical monitoring for IRC 003 and IRC 004; to support the TV005 dengue study in Bangladesh; to support studies through the La Red clinical network in Mexico; and statements of work (SOWs) were developed to support clinical monitoring of the Predict studies in China, Taiwan, and South Africa. The vendors for these efforts are to be determined, with work starting in FY2017.

Financial Management

The CCS group provides support to the CMRP Financial Management Group (FMG) with budget preparation and spending predictions utilizing the CMRP Financial Management SharePoint site. During the reporting period, close-out of the FY2015 DCR annual budget was completed, as well as four rounds of spending predictions for FY2016. Budget preparation for FY2017 also occurred.

Travel, Conference, and Meeting Coordination

The CCS group provides travel coordination for nongovernment and CMRP employees involved in major NIAID initiatives. The group coordinates international and domestic meetings, conferences, and training for nongovernment participants collaborating on many long-term, clinical research initiatives. The services include arranging visits by domestic and/or foreign scientists and officials to various national and international locations to attend meetings, conferences, planning sessions, and program discussions; developing detailed travel itineraries; providing guidance to U.S. and foreign travelers in obtaining passports and/or visas; arranging ground transportation as necessary; arranging hotel or other lodging accommodations; making direct contact with the host and the traveler to ensure all arrangements are mutually understood; and providing reimbursement upon receipt of an expense statement for appropriate expenses relating to travel.

Building Management

The CMRP CCS group provides support to a leased building in Frederick, MD. This building houses CMRP employees working in support of NIAID DCR. Staff members guide and coordinate all areas of lease oversight, facility maintenance, facility renovation and design, staff relocations, problem resolution, preventive maintenance schedules, and coordination with outside vendors. Over the last year, several facility projects required extensive support, including the transition of the existing phone system and infrastructure to Voice over Internet Protocol (VoIP), office space reconfigurations to add additional work stations in support of new work efforts, hardware upgrades to the existing card reader system, and the transition of over 350 building light fixtures to LED technology. The VoIP project required several months of planning, which included deploying 145 phones, coordinating nine training sessions, and scheduling a weekend cut over to ensure minimal impact to staff.

Due to the light upgrade, NIAID DCR will no longer pay a portion of the electric bill for the leased facility located in Frederick, MD.

Project/Program Management Support

The CCS group provides project/program management services for various domestic and international DCR initiatives. This includes operational and logistical management of subcontracts, site renovations, shipping, inventory, travel, foreign insurance, budgets, and oversight of all aspects of the HHS Efficient Spending Policy. The clinical project manager and program managers monitor project plans and timelines, develop progress reports, assess project issues, and develop resolutions to meet productivity, quality, and customer objectives.

In response to evolving threats in various countries, the U.S. Department of State (DOS) announced in December 2015 the requirement for increased security trainings for U.S. government personnel who travel to certain foreign locations. Although this training is not required for contractors, CMRP determined that it was imperative for those staff members and subcontractors who travel to international locations. In March 2016, CMRP made the High Threat Security Overseas Seminar (HTSOS) online course a mandatory training requirement. This eight-module online training course was rolled out in four waves to CMRP international travelers beginning first with the most critical travel areas and frequent travelers. CCS coordinated the training process with the Learning and Professional Development (L&PD) group and supported a tracking mechanism to ensure all necessary staff and subcontractors completed the training by September 2016.

Technical and Scientific Support

CCS staff members also provide operational leadership and technical and scientific support to NIAID's Collaborative Clinical Research Branch (CCRB). This includes oversight of special projects and initiatives, and disseminating research information to the clinical community. A CMRP physician and clinical project manager support CCRB activities by providing technical expertise for the implementation and management of research strategies, developing and teaching sound clinical concepts related to infectious disease research, and by presenting and publishing valuable information to the research community. The support provided by these specialized persons is discussed in the CCRB section of this report.

Administrative Support

The CCS group's administrative staff members manage program schedules, coordinate meetings, prepare agendas and disseminate meeting minutes, make conference arrangements (local and international), schedule guest speakers, coordinate training sessions, help prepare domestic and foreign travel packages, track action items related to branch initiatives and project milestones, and coordinate with project teams to compile and distribute information as directed.

Repository Support

The CCS group's program manager and senior program coordinator provide support and management oversight for a subcontract with Fisher BioServices. This subcontractor provides the storage and shipment of clinical research material to domestic and international locations in support of DCR. This includes communication with the principal investigator (PI), preparing request forms, arranging shipments, tracking shipments, assuring that template data is received/reviewed, and making sure that all required documents are completed and returned. Staff also monitors the budget and provides a monthly estimate at completion.

Courier Support

Due to the ongoing Ebola response, additional courier runs continued in FY2016 to the embassies of Liberia, Sierra Leone, and Guinea, as well as to the U.S. Department of State. The delivery of medical supplies from the National Institutes of Health (NIH) Pharmacy and Integrated Research Facility (IRF) to Leidos Biomed Transportation department was also supported by CCS staff.

Office of the Director

Support Provided by the Clinical Monitoring Research Program

HIV Clinical Research in West Africa

In late 2014, under the portfolio for emerging/re-emerging viral hemorrhagic fevers (VHF) and other infectious diseases, the National Institute of Allergy and Infectious Diseases (NIAID) initiated a randomized, controlled vaccine trial, the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL I), in West Africa to test the safety and efficacy of two Ebola vaccine candidates. During these trials, NIAID noted a 10 percent prevalence of human immunodeficiency virus (HIV) in female participants.

As the primary U.S. government institute focused on HIV/acquired immune deficiency syndrome (AIDS) research, NIAID is committed to conducting the research necessary to successfully end the global fight against HIV/AIDS. To support this mission, DCR has requested the services of CMRP to develop and manage a portfolio of multi-year HIV/co-infections clinical research studies tailored to HIV/AIDS in West Africa. Building upon the partnerships and capacity established for VHF clinical research efforts, CMRP will support scientific/clinical, technical, and program/project oversight for the development, rapid deployment, and management of an HIV clinical research network in West Africa. While not intended to be an all-encompassing list, such studies may be designed to: (1) better characterize the epidemiology of HIV infection in Liberia and the region of West Africa; (2) assess the different responses to therapy and the impact of co-infections on disease progression; and (3) seek to determine the role of HIV infection in accelerating chronic illnesses, such as cardiovascular and renal diseases.

A strategic planning meeting, which also served as the kick-off, was held in November with NIAID leadership to outline initial guidance for this effort. Another strategic planning meeting was held in Monrovia, Liberia, in March with NIAID leadership and Liberian partners, primarily for the VHF portfolio, and it included discussions about the initial data on HIV prevalence in Liberia and the potential for HIV protocols in the region. Ongoing meetings with NIAID and Leidos Biomed leadership are informing infrastructure planning and other preparatory work.

Once the HIV Clinical Research in West Africa portfolio reaches higher priority, Leidos Biomed/CMRP will engage resources and partnerships established under the VHF portfolio to support this project.

FLU-PRO (Symptoms Scale)

To support NIAID DCR's effort to provide a standard measurement of influenza symptoms for use in clinical studies, Leidos Biomed developed the Influenza-Patient Reported Outcomes (Flu-PRO) scale. A standardized, validated patient-reported outcome (PRO) measure, created according to accepted scientific standards, did not exist. Earlier instruments lacked patient input about relevant symptoms or did not undergo formal evaluation in terms of measurement properties. Additionally, severity indices for influenza were based on admission to a hospital or an intensive care unit. Because admission criteria can vary between geographic sites, such measures lacked standardization. The goal of the Flu-PRO initiative was to decrease variability, increase validity, and enable comparisons between studies by creating a measurement tool that could be directly assessed from patients on a daily basis.

The development of Flu-PRO consisted of three stages, all of which were completed in FY2015 (enrollment, data collection, site close-out, data analysis, and final report). During FY2016, CMRP continued project oversight with activities focused on preparing publications and closing out subcontracts. The manuscript of Stage 1 and 2 data was published in *BMC Infectious Diseases* in January 2016, and a poster titled "Evaluation of the Performance Properties of the Influenza Patient-Reported Outcomes Instrument (Flu-PRO)" was presented at the International Society of Pharmacoeconomics and Outcomes Research (ISPOR) 21st Annual International Meeting in May 2016. A manuscript of the Stage 3 data is in development. All Leidos Biomed subcontracts related to this project were reviewed, audited, and formally closed. Flu-PRO can be used in epidemiological studies of the natural history of influenza, evaluation of influenza treatment or prevention intervention outcomes, and in combination with other variables to develop a standardized severity index for influenza.

Leidos Biomed copyrighted the Flu-PRO instrument and licensed its use to Gilead Sciences for use in a respiratory syncytial virus (RSV) clinical study, to MedImmune for use in RSV and influenza studies, to Nanotherapeutics, Janssen, and Micron for influenza studies, and to WCCT for influenza challenge studies. This effort aims to validate the Flu-PRO instrument for influenza as well as a different disease with similar symptoms. The agreements allow for sponsors to develop Flu-PRO in additional languages and return to Leidos Biomed for further evaluation.

Respiratory Virus and Other Emerging. Re-emerging, Infectious Diseases

Research supported and conducted by NIAID strives to understand, treat, and ultimately prevent the myriad of infectious, immunologic, and allergic diseases threatening the health of millions of people in the United States and around the world. Against a background of established infections, epidemics of new and old infectious diseases emerge. Emerging/ re-emerging and related respiratory (ERRR) viruses causing diseases such as severe acute respiratory syndrome (SARS), influenza, and Middle East respiratory syndrome coronavirus (MERS-CoV) are of particular concern given their significant morbidity and potential for rapid geographic spread.

One of DCR's primary objectives is to provide an effective rapid response of clinical research activities in support of emerging disease priorities as set forth by the HHS, the National Institutes of Health (NIH), and other agencies. This objective supports a better understanding of the diseases and therapeutic options and aims to improve medical outcomes for patients afflicted with the ERRR viruses.

For these reasons, NIAID's DCR requested that CMRP provide support to establish infrastructures related to study conduct, including innovative methodologies associated with patient recruitment and the monthly follow-up of subjects, site initiation and activation, establishment of manuals of operations, regulatory oversight, Data and Safety Monitoring Board (DSMB) oversight, data management, clinical trials monitoring, operations management, biostatistics, communications, training, project management, logistics management, laboratory and repository operations, and study agent management and oversight.

Each of the initiatives highlighted below are of critical importance in the development of a prepared global clinical research community poised to respond to the next emerging infectious disease challenge.

During FY2016, CMRP provided scientific and technical management and oversight for NIAID DCR to the following collaborations and networks:

- **NIAID Influenza Research Collaboration (NIRC) –**
  NIRC studies: evaluated the safety of using human plasma containing high-titer influenza antibodies in addition to antiviral medications in treating subjects with severe influenza (IRC 002); evaluated whether combination therapy with three antivirals (compared to one antiviral) will help symptoms resolve faster and with fewer complications (IRC 003); sought to understand whether subjects on Tamiflu show decreases in the amount of virus detected in the nose or throat, and whether the change in the amount of virus is associated with changes in symptoms (IRC 004); and compared the efficacy and safety of anti-influenza immune plasma (IRC 005).
- **International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) –**
  INSIGHT research: studied outpatient subjects with confirmed or suspected influenza (FLU 002 Plus); studied subjects hospitalized with confirmed

or suspected influenza (FLU 003 Plus); studied polymorphisms in immune response genes and other genetic variants that may be associated with an increased risk of disease (INSIGHT 004); and tested the safety and effectiveness of an antibody against flu in people who are hospitalized for severe flu (INSIGHT 006).

- **South East Asia Infectious Disease Clinical Research Network (SEAICRN) –** SEAICRN studies: examined the etiology of sepsis in Indonesia (SEA050); and identified known and unknown viral pathogens in undiagnosed patients enrolled in SEA050 (SEA050 substudy).
- **Mexico Emerging Infectious Disease Clinical Research Network (La Red) –** La Red Network studies: evaluated the safety, effectiveness, and tolerability of nitazoxanide in treating severe acute respiratory illness in people who are hospitalized (NTZ-SARI); determined the causes of and identified increases in influenza-like illnesses (ILI 2014); evaluated culture negative severe influenza-like illness (CN-SARI); and evaluated the cause of Zika-like illnesses in southern Mexico to determine if these illnesses are from Zika, chikungunya, dengue, or other viruses (Zik01).

On March 21, 2016, CMRP received notification from the NCI Management Operations Support Branch (MOSB) explaining that non-respiratory related studies that are considered emerging infectious diseases (e.g., Zika virus) fall within the intended scope of work under the Respiratory Viruses Research IDIQ TO 15 and can therefore be conducted within the La Red Network (Zik01 study).

During the reporting period, CMRP provided technical, clinical, scientific, and programmatic/ project management oversight of the clinical research studies described above. More specifically, CMRP provided support to: (1) characterize the natural history of influenza, influenza-like illness, and other respiratory diseases across several different geographic areas and resource settings, and encompass different severities of disease using models and methodologies that can be adapted rapidly; (2) evaluate novel therapeutic platforms and paradigms such as innovative plasma treatments that can be used to respond to emerging infections; and (3) ensure flexibility and positioning so that both natural history and therapeutic studies are adaptable to rapidly respond to new and urgent emerging diseases.

CMRP staff who supported the efforts listed above during FY2016 included 1 medical affairs scientist, 3 clinical project managers, 2 associate clinical project managers (1 hired in April 2016), and 1 administrative assistant.

Detailed information about each of these efforts can be found in their respective sections of this report.

NIAID Influenza Research Collaboration (NIRC)

The NIAID Influenza Research Collaboration (NIRC) is an NIH/NIAID-sponsored clinical trials network dedicated to finding new treatments for seasonal and pandemic flu.

During the reporting period, CMRP supported activities for the following NIRC studies:

IRC 002 ("Randomized, Open-Label, Phase II, Multicenter Safety and Exploratory Efficacy Study of Investigational Anti-Influenza Immune Plasma for the Treatment of Influenza")

The IRC 002 protocol was launched in December 2010. In March 2015, the protocol completed enrollment, reaching an enrollment/ randomization number of 98 (with a goal of 100 infused subjects). Site monitoring was initiated in order to conduct close-out visits, and plasma return was also completed, with sites returning all unused units to the repository. The study closed in March 2015, the database was locked, and the final analysis was completed during this reporting period. A manuscript is currently being drafted for publication.

IRC 003 ("Antiviral Efficacy of Combination Antivirals in the Treatment of High-Risk Outpatient Influenza")

IRC 003 focuses on enrolling subjects who are at risk of developing severe influenza based on criteria set by the Centers for Disease Control and Prevention (CDC). The purpose of the study is to evaluate whether combination therapy with three antivirals (compared to the standard of care, one antiviral) will help symptoms resolve faster and with fewer complications.

The IRC 003 protocol was launched in January 2011 in the U.S., followed by Australia in August 2011 (closed in FY2014), Mexico in February 2012, Thailand in June 2012, and Argentina in July 2012. During the 2015–2016 flu season, 247 subjects were enrolled and 201 randomized at the U.S. and international sites, bringing the total number of subjects enrolled to 880 and randomized to 634. Additional sites were sourced in the U.S. and added to the IRC 003 study site roster.

Due to low numbers of IRC 003 study agent inventory, a process was developed during FY2015 to transfer study agent between sites within the U.S. This process allowed for redistribution of study agent from lower-recruiting or closing sites to new or higher-recruiting sites. During FY2016, the following transfers were completed: 14 in the U.S.; five in Argentina in preparation for import into the U.S.; and one in Mexico.

During FY2016, the clinical project manager oversaw the 60-month stability testing of the IRC 003 investigational product. Documentation of the stability results was distributed to the Regulatory Affairs department for inclusion in the IRC 003 Annual Report to the FDA. Sites were informed that the retest date was being extended to June 30, 2016. CMRP staff generated new retest date labels, tamper seals, and over-labeling instructions that were shipped to all sites prior to the original retest date. After all available documentation was provided and questions were answered, the Thai FDA did not approve the extension of the retest date for IRC 003. As a result, the study was closed in Thailand in March 2016, earlier than in the U.S. (April 2016).

For IRC 003, the nasal specimens tested at the Naval Health Research Center (NHRC) were displaying negative influenza results at a high rate for specific sites. Further review and analysis with an expert at AVR Laboratories demonstrated very low housekeeping gene expression, which determined that improper collection and/or mixing of the swab in Universal Transport Medium (UTM) at sites was the cause of the negative test results. To combat this, the eight sites identified with a higher-than-expected rate of negatives, coupled with new sites activated during FY2016, underwent the Virology Quality Assurance process. This involved the retraining of site staff and a collection of nasal specimens from volunteers in order to approve the re-opening of enrollment screens for participation in the study. To date, all eight sites have successfully completed this process. Additionally, vortex mixers were supplied to sites in order to aid in the proper mixing of swab specimens.

A new challenge occurred when false positive flu tests were being reported at NHRC, yet the specimens exhibited the presence of housekeeping genes, which no longer implied improper collection at the sites. The primary issue being addressed in March 2016 was the high percentage of negatives showing up. It was confirmed that the samples in UTM being sent to NHRC were low in virus and not assay failures or strain variants. The issue was related to a difference in viral load between the samples analyzed with Sofia kits and the UTM samples. Because the Sofia samples use the entire swab applied directly to a small volume of diluent, they are as much as 1,000 times more concentrated than the UTM samples. A new quality control test was designed to review the potential cause of the false positives, testing Sofia samples in parallel with their paired UTM samples. The analysis is not yet finalized. In order to compensate for the sites submitting false negatives, the medical affairs scientist recommended that the enrollment and randomization goal be increased, and that recommendation was approved by the Data and Safety Monitoring Board. Additionally, due to the closure of study operations in Argentina in November 2015, 58 IRC 003 study agent kits were exported from Argentina to the United States for domestic distribution. These kits were utilized to meet the increased enrollment and randomization goal rather than incurring costs for manufacturing new study agent kits.

The clinical project manager/lab operations manager scrutinized the reports tracking IRC 003 and IRC 004 samples weekly for missing or erroneous data in conjunction with the repository and customer. Several report revisions have been engineered by the subcontractor due to discrepancies. The clinical project manager/lab operations manager created a master query file that is updated weekly, to include new queries for site resolution, as well as documentation of all resolved queries for historical tracking purposes. The timeline to ship virology specimens to the NHRC and obtain reported results was also monitored. Monthly conference calls were held to review pending discrepancies, queries, updates to the database, and other solutions to improve data quality. As a result, communication between the subcontractor and team has greatly improved, and responsiveness has increased.

On April 30, 2016, the study, having met its enrollment and randomization goal, was closed. For IRC 003, the original goal was to have 560 subjects randomized, which was increased to "up to 700" subjects randomized. As mentioned above, this was later increased in order to compensate for sites that submitted false negatives. Notably, and despite the late start of the flu season, the increased amount for subjects randomized was reached; a total of 634 subjects were randomized. Final data analysis is expected to occur during FY2017.

IRC 004 ("Antiviral Efficacy of Oseltamivir versus Placebo in Low-Risk Outpatient Influenza")

IRC 004 seeks to understand whether subjects on Tamiflu show decreases in the amount of virus detected in the nose or throat, and whether the change in the amount of virus is associated with changes in symptoms. Subjects at low risk for developing complications were randomized to receive either Tamiflu or a placebo.

The IRC 004 protocol was launched in January 2012 domestically, and then by Thailand in June 2012 and Argentina in July 2012. During this reporting period, three sites in Thailand were

actively participating in IRC 004 (all other study sites have been closed). Across all participating sites, 443 subjects were enrolled and 338 were randomized.

During the next flu season, the study will be re-started in the U.S. In support of this, CMRP staff worked closely with vendors and the operations team to identify and assess potential new sites in addition to existing IRC 003 sites that may be interested in participating in IRC 004 in the U.S. Since there are some site duplications among studies, laboratory kit supply inventories were assessed to determine which sites may need new inventory of kits and which may need replacement parts for any items expiring. CMRP staff requested a revised budget from the subcontractor in anticipation of U.S. involvement and a continuation of IRC 004. A request for proposal was written and issued to identify potential vendors to manufacture a new lot of study agent for the study.

IRC 005 ("Randomized Double-Blind Phase 3 Study Comparing Efficacy and Safety of Anti-Influenza Immune Plasma") - IRC 001B ("Collection of anti-influenza plasma at community blood banks in support of IRC 005")
Anti-influenza plasma is used in both a treatment study (IRC 005) and manufactured into intravenous immunoglobulin for use in INSIGHT 006.

In an effort to increase plasma collection and to lower the costs of plasma units to the U.S. government, plasma collection was transitioned in FY2015 from donor-directed (screening a population to find appropriate donors, then collecting plasma from those donors) to screening units collected as routine at community blood banks to find units that meet the study requirement of high-titer anti-influenza antibodies. This program functions with the Mississippi Valley Regional Blood Center in Iowa, the Gulf Coast Regional Blood Center in Texas, and the Memorial Blood Bank in Minnesota.

To date, more than 3,000 units of plasma have been collected through this mechanism. At the end of the 2015–2016 northern hemisphere influenza season, 96 plasma units, totaling approximately 28 L plasma, were identified and shipped to Emergent BioSolutions for intravenous immunoglobulin (IVIG) manufacturing.

IRC 005 was implemented during FY2016 in July. Through the end of the 2015–2016 northern hemisphere influenza season, IRB approvals had been obtained at 29 sites in the U.S. with subcontracts fully executed at 24. An additional five sites do not require subcontracts (NIH and Department of Defense sites). The first site was opened in December 2015, and the first subject was enrolled in January 2016. In total, 55 subjects have been enrolled, with 37 subjects randomized during the 2015–2016 flu season.

During FY2016, database development of a plasma unit Return Module is under way to allow sites to return any unused, unexpired plasma to the repository for future manufacturing of IVIG. Three blood banks began the collection of plasma units in August 2015 for high/low-titer testing and subsequent request and distribution to the repository for storage. As sites were activated, plasma shipments were distributed in late fall 2015 using a new database developed by subcontractors for real-time inventory, distribution, and designation status. The clinical project manager worked with the protocol team to design serology and virology kits and labels to be provided to all activated sites. In addition, the clinical project manager worked closely with the protocol team and the client to design and test the new automated plasma ordering database, as the only member of Leidos Biomed to be unblinded throughout the course of the study. The IRC 005 Investigator Meeting was held December 3–4, 2015, in Washington, D.C., with participants in attendance from NIAID, Leidos Biomed, SSS International Clinical Research, and all participating clinical sites.

During FY2016, plasma was collected for use in IRC 005 under IRC 001B. To promote cost savings, plasma that is not utilized for the IRC 005 study will reduce quantities needed from Emergent BioSolutions and decrease costs for manufacturing IVIG.

In support of these studies, CMRP maintained research subcontracts, consulting agreements, purchase orders, and one blanket purchase agreement during FY2016 to provide:

- Coordination activities to IRC 003 (SSS).
- Clinical monitoring support to IRC 003 and IRC 005 (DP Clinical, Inc.).
- Clinical monitoring support for IRC 003 and IRC 004 international sites (Pharmaceutical Product Development [PPD]).
- Support to NHRC for virology testing in IRC 003 and IRC 004 (AVR Laboratories)
- Sample collection kits to IRC 003, IRC 004, and IRC 005 (Therapak).
- Good Manufacturing Practice (GMP) manufacturing, storage, and worldwide distribution for IRC 003 and IRC 004 (Fisher Clinical Services).

During FY2016, per a change in Leidos Biomed's prime contract with NCI, research subcontracts and agreements awarded under the Operations and Technical Support (OTS) contract were modified and

re-awarded under the new IDIQ contract (NIRC is under TO 15 of the IDIQ). CMRP staff worked with Research Subcontracts to award new TOs with multiple vendors to ensure there were no delays in the work performed.

The CMRP medical affairs scientist, who functions as lead associate investigator for implementing all of the NIRC treatment studies at the NIH Clinical Center, provided ongoing technical leadership to the projects. Activities included meeting with Principal Investigators (PIs); revising protocols; serving as the contracting officer technical representative (COTR) on several research subcontracts; and providing scientific guidance related to study procedures, subject enrollment, inclusion, and exclusion criteria; and global influenza status.

The clinical project manager/laboratory operations manager was heavily involved in the day-to-day administrative management of several research subcontracts, routinely reviewing and approving monthly reports and invoices, research subcontractor travel requests and trip reports, as well as monitoring budgets, budget modifications, expenditures, and end-of-year forecasting.

The administrative assistant managed the administrative aspects of the program, including producing meeting minutes, generating purchase requests, overseeing the acquisition process flow, pre-processing vendor invoices, managing and updating clinical site lists for international shipping and CDC import permits, tracking biospecimen shipments and notification lists, organizing and maintaining electronic study files, and compiling invoicing spreadsheets.

International Network for Strategic Initiatives in Global HIV

NIAID requested that Leidos Biomed facilitate the conduct of clinical trials through the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT). Leidos Biomed awarded a Basic Ordering Agreement (BOA) to the University of Minnesota to allow for multiple influenza-like illnesses (ILIs)/Emerging Infectious Diseases TOs.

During the reporting period, CMRP staff supported the following initiatives for the INSIGHT Network:

- **Flu 002 Plus**, a natural history study of outpatient subjects with confirmed or suspected influenza.
- **Flu 003 Plus**, a natural history study of subjects hospitalized with confirmed or suspected influenza.
- **INSIGHT 004**, a genomics sub-study for the FLU 002 PLUS, FLU 003 PLUS, and INSIGHT 006 studies.
- **INSIGHT 006**, an IVIG to treat severe influenza.

CMRP provides ongoing management to the following research subcontracts and a new consultant agreement (executed in FY2016) to support the INSIGHT studies:

- University of Minnesota – provides support for all INSIGHT network protocols.
- Sage Analytica – provides support for INSIGHT manuscript development arising from FLU 002 Plus and FLU 003 Plus.
- Henry M. Jackson Foundation – conducts hemagglutination-inhibition (HAI) titer testing.
- Professor Sean Emery, Ph.D. – provides consultant support to treatment research needs for poor and developing countries and research initiatives, aiding in the defining optimum methods to design and implement rational and responsible clinical research.

Per a change in Leidos Biomed's prime contract with NCI, research subcontracts and agreements awarded under the OTS contract were re-awarded under the new IDIQ contract (INSIGHT is under IDIQ TO 15). CMRP staff worked with Leidos Biomed Research Subcontracts to execute new agreements with the University of Minnesota for the continued administration of the studies. Flu 002 and Flu 003 were revised to allow for enrollment of participants with other targeted non-influenza (TNI) respiratory viruses of public health concern and were renamed Flu 002 Plus and Flu 003 Plus.

During FY2016, subject enrollment met the following numbers in these studies: 2,003 participants in Flu 002 Plus, 637 participants in Flu 003 Plus, 1,991 participants in INSIGHT 004, and 51 enrolled in INSIGHT 006.

To best utilize the data collected from the INSIGHT studies, Leidos Biomed executed a research subcontract with Sage Analytica in FY2015. During FY2016, Sage Analytica continued to provide scientific expertise and work collaboratively with INSIGHT investigators and other influenza investigators to generate study concepts and submit them for review to the INSIGHT and the NIAID Scientific Steering Committee (SSC). A new agreement was executed with Sage Analytica under IDIQ TO 15 in April 2016. The SSC approved the following concept, which was developed into a manuscript titled, "Using INSIGHT Data to Rapidly Assess Key Clinical and Epidemiological Characteristics of a Future Pandemic." The information from this publication was also used for an abstract at the Options (IX) for the Control of Influenza Conference held August 24–28, 2016 in Chicago.

Sage Analytica addressed comments from the SSC on the "Influenza vaccine effectiveness against ILI and the Influenza B Linage Distribution" publication concept. Once reviewed again by the SSC, the concept will be approved or rejected. Additional concepts are under development for future publication activities.

Using the specimens and data collected under the FLU 003 Plus protocol, the INSIGHT Site Coordinating Center (SCC) approved a project entitled, "Are Increased Kyn-to-tryp Ratios as a Marker of Enhanced Indoleamine-2, 3-dioxygenase (IDO) Activity Associated with Poor Clinical Outcomes (PCR) in Adults Hospitalized with Influenza." This case control study explored the association of IDO activity—as measured by the kyn-to-tryp ratio in Flu 003 Plus patients with PCR-confirmed influenza H1N1 who died and/or progressed to mechanical ventilation after enrollment versus matched controls who did not die and/or progress to mechanical ventilation. During FY2016, the laboratory and biostatistical analysis was performed at the University of Minnesota and a publication was submitted for publication.

Intravenous Immunoglobulin Outcome Study

Enrollment continued for the global INSIGHT 006 Flu-IVIG, "Anti-Influenza Hyperimmune Intravenous Immunoglobulin Clinical Outcome Study" (INSIGHT 006); there are currently 76 subjects enrolled. The IVIG Outcome Study is a double-blind, placebo-controlled, randomized Phase II trial of Flu IVIG and Standard of Care (SOC) versus placebo and SOC in patients 18 years of age and older who are hospitalized with influenza to compare the percent of patients who died, or who remained hospitalized, at day seven for patients assigned Flu IVIG versus saline placebo. There are currently 25 sites open for this study: U.S. (17), Australia (1), U.K. (3), Spain (3), and Denmark (1).

CMRP managed a research subcontract for the manufacturing of an additional lot of IVIG. Human plasma with high titers for anti-influenza antibodies were collected by a subcontractor and from the IRC blood banks. The subcontractor, Emergent BioSolutions, used that plasma to manufacture concentrated IVIG. To ensure that the appropriate plasma units were used, an influenza HAI assay was conducted; CMRP also managed a research subcontract with the Henry M. Jackson Foundation to provide a laboratory technician who performed this HAI testing at the Naval Medical Research Center. The plasma units from Emergent BioSolutions and the IRC blood banks with the highest influenza antibody titers to the relevant circulating influenza strains, which can change each season, were identified and shipped to Emergent BioSolutions for the manufacture of IVIG.

For the additional lot of IVIG manufactured during FY2016, CMRP provided administrative oversight and scientific and technical leadership, including technical management of the research subcontract; establishing manufacturing, drug substance, and drug product parameters and release criteria; establishing stability testing; and providing regulatory support needed to use this product in the treatment study.

CMRP collaborated with key staff at the Leidos Biomed Vaccine Clinical Materials Program (VCMP) to provide GMP expertise. Specifically, VCMP provided assistance and input for the Emergent BioSolutions quality agreement and statement of work (SOW), which outlined technical GMP standards and requirements for IVIG. VCMP also reviewed all manufacturing batch records (in-process and control records, out of specifications results, environmental monitoring deviations, and investigations/supporting documentation of all observation/comments made) to ensure the IVIG lot was produced in compliance with GMP regulations. At the request of VCMP, CMRP initiated a subcontract with the National Science Foundation (NSF) Health Sciences Pharma Biotech Consulting to perform a GMP compliance audit of the manufacturing facility used by Emergent BioSolutions for IVIG. The GMP audit took place in June 2016 and noted two observations: (1) uncontrolled access in the clean room facility, and (2) filling at risk with respect to pre-filtration bioburden. Emergent will perform a quality assessment on these findings and provide a corrective and preventative action report. NSF found that Emergent BioSolutions was a high-quality facility and is suitable for clinical trial material manufacturing in the U.S. and Europe.

During the reporting period, CMRP and the Leidos Biomed Subcontracts Department modified the research subcontract with Emergent BioSolutions to manufacture another batch of IVIG under the IDIQ contract in FY2017.

An ongoing challenge with the Flu studies during FY2016 was over-enrollment beyond the yearly projected amounts. This, coupled with the quarterly payment structure to the International Coordinating Center (ICC)/SCC, caused the University of Minnesota to incur expenses beyond the approved annual budget. To mitigate this, monthly progress reports began to include real-time monthly enrollment numbers for each ICC/SCC (as they are outlined in the budget); these numbers were compared with the anticipated enrollment amount. The CMRP clinical project manager and INSIGHT operations staff were able to make decisions about enrollment prior to having a budget overrun. When enrollments encroached the budgeted enrollment amounts, CMRP worked with the University of Minnesota to control enrollment through the following methods: reduced enrollment at sites that historically

enrolled high numbers of influenza-negative patients; modified the agreements to realign the budget to the projected enrollment amounts; and/or halted enrollment. The CMRP clinical project manager provided monthly enrollment updates to all stakeholders to ensure an early intervention, if required.

Reducing enrollment while still ensuring a diverse mixture of participants both geographically and temporally was challenging. To combat this, the INSIGHT team placed higher emphasis on enrollment into Flu 003 Plus, which allowed the team to make decisions that continued the required diversity of participants enrolled.

Execution of the INSIGHT TO under the IDIQ took longer than expected due to contractual requirements necessitating new agreements rather than the modification of existing agreements. This delay resulted in the need to charge approximately $2.5 million of unanticipated expenses to the ERRR OTS cost center. To mitigate the financial impact, Flu expenses incurred in the Southeast Asia region (Thailand and Australia) were charged to the SEAICRN cost center, as the work completed in this region was within the scope of work for the SEAICRN Yellow Task (YT). Additional prior year funds were requested from NIAID DCR and added to the ERRR OTS cost center to cover the budget overrun.

During this reporting period, the European Union (EU) updated its regulation on the importation of investigational new drugs and drug products. The new regulations affected the documentation that must accompany drug shipments to any country within the EU. To meet these new regulations by September 2016, Emergent BioSolutions provided the following documentation to Almac for all IVIG lots: the product specification file, the Transmissible Spongiform Encephalopathy/Bovine Spongiform Encephalopathy declaration, recent audit reports and corrective and preventive actions (CAPAs), and the risk assessment for each component used in the manufacturing process.

South East Asia Infectious Disease Clinical Research Network

The South East Asia Infectious Disease Clinical Research Network (SEAICRN, or "the Network") was established in 2005 to address avian influenza, although it has increased its scope to include other emerging infectious diseases in the region. This research collaboration is of the highest priority for HHS, NIH, and NIAID, and is one of several special projects for NIAID DCR that fosters international, collaborative, clinical research. In FY2013, CMRP facilitated a competitive solicitation and awarded several multimillion-dollar research subcontracts to Family Health International 360 (FHI 360) under a BOA. The research subcontracts established the operational infrastructure to support SEAICRN's research portfolio and provided technical support and administrative assistance to the network and site management for the clinical research sites in Thailand and Vietnam.

CMRP focused resources on addressing and resolving the logistical challenges of conducting international clinical research; these challenges include complying with the multiple and varying regulations of different countries, identifying and improving unequal levels of readiness to conduct research among sites, and overcoming language barriers.

CMRP staff supported the following initiatives for SEAICRN in FY2016:

- Sepsis study (SEA-050) – a natural history study of the etiology of sepsis in Indonesia.
- SEA-050 sub-study – a viral metagenomics analysis of undiagnosed sepsis patients enrolled in SEA050.

CMRP maintained two research subcontracts and one consultant agreement with the following vendors to support the SEA-050 studies:

- FHI 360 provided technical, operational, and administrative support, and overall coordination of SEAICRN activities.
- PPD provided clinical monitoring support services.
- Sean Emery, Ph.D. is a consultant supporting treatment research needs for poor and developing countries, research initiatives, and aiding in defining optimum methods to design and implement rational and responsible clinical research. This consulting agreement was executed in FY2016.

A clinical project manager manages the SEAICRN project. During FY2016, this CMRP staff member supported the following activities of the Network: ensured necessary communications were provided or directed to the proper stakeholders; tracked participant enrollment and overall trial timeline to meet the project expectations; maintained overall budget information; reviewed and approved invoices and monthly progress reports from the subcontractor; participated on weekly project status teleconferences with leading domestic and international stakeholders; and provided expert advice for operational and technical risks to ensure adherence to scope, budget, and timeline.

Sepsis Study (SEA-050)

SEA-050 is a natural history study that tries to determine the causes of community-acquired sepsis and severe sepsis in adults and pediatrics across Southeast Asia. During this reporting period, Leidos Biomed, FHI 360, and the SEAICRN partners completed study enrollment (750 participants in Thailand and Vietnam and 82 in Indonesia—for a total of 1,582 participants). An ongoing challenge with managing the study was ensuring that

enrollment was even across seasonal variations and during local holidays. Removing the monthly enrollment caps allowed for the enrollment target to be met in Thailand and Vietnam several weeks ahead of time in mid-November 2015.

During FY2016, clinical trials site specialists traveled to each site to ensure all sepsis study activities were completed and documented. The protocol was amended at each site to include open access of study data, all of the Thailand and Vietnam cases were adjudicated, the Thailand and Vietnam database was locked, and the study statistical analysis outlined in the Statistical Analysis Plan was completed. In addition, the clinical trials site specialists supported the Indonesia Research Partnership on Infectious Disease (INA-RESPOND) with conducting the study at four sites in Indonesia; a total of 82 patients were enrolled.

FHI 360 continued maintenance of the Network's SharePoint site for housing all documents through the end of their contract in April 2016. All documents from SEA-050 were provided to Leidos Biomed, the Network Director, and the NIAID Project Officer. The historical documents housed on the SEAICRN SharePoint site were provided to the Network Director for inclusion on the SEAICRN website, which is maintained by Oxford personnel.

The FHI 360 biostatistical team conducted the final analysis on the data and provided draft data tables for the publication. All information used for the data analysis will be uploaded to FigShare by the Network Director. All raw data, as well as publication tables and figures, were provided to Leidos Biomed, the Network Director, and the NIAID project officer.

The Governing Board (GB) and Executive Committee (EC) met in Bangkok, Thailand in January 2016. In addition to preliminary data from the sepsis study, the group decided to disband the EC since there will be no additional Network-wide trials conducted. The group also decided to expand the GB to include the Network Director and, when necessary, to include other Network and non-Network individuals who could bring insight or experience for proposed discussion topics. The exact mechanisms for this new GB will be determined and documented by the new GB Chair.

FHI 360 supported Indonesia in initiating the database and providing training to site study staff, retrieving data queries, tracking participant enrollment, and completing the case report form in order for it to be disseminated to key stakeholders. FHI 360 also assisted in documenting the destruction of the remaining SEA-001 specimens stored in the Thailand and Vietnam repositories, and submitted the "Genotype Replication of Genetic Variants Associated with Severe Influenza" (EXOM Study) to the Ministry of Public Health IRB for approval.

In the interest of reducing costs associated with travel, the CMRP clinical project manager attended meetings of less than two days' duration through teleconferencing. This option was also extended to FHI 360's project manager living in the U.S. Whenever possible, the Southeast Asia–based project manager attended the face-to-face meetings, which reduced travel and labor costs by nearly $25,000. Additionally, to reduce overall travel costs, the SEAICRN GB and EC meetings have occurred during the same week and at the same venue.

Obtaining invoices from the vendor in a timely fashion was a challenge during FY2016. Invoicing was done centrally at the FHI 360 headquarters in the U.S. The financial group needed to have all receipts of expenses sent from the field and then reconciled in their billing system. Aside from the language barrier, the difference between Leidos Biomed's contract polices and FHI 360's policies caused a delay. For example, unlike FHI 360, Leidos Biomed required receipts for expenses under $50; FHI 360 complied by creating a document to handwrite receipts. Once submitted, oftentimes the U.S.-based group would need to have field staff to answer any invoicing questions that arose, which further delayed the approval process. These invoicing issues also delayed the close of FHI 360's overall contract. In order to remedy the situation, CMRP held weekly conference calls with the project manager and financial staff and obtained updates on the status of the invoicing problem.

Additionally, keeping the EC members engaged continued to be a challenge throughout the project. Although the overall committee structure was changed to include new country representatives and senior advisors, the engagement of the members continued to decline.

SEA-050 Sub-Study

During the reporting period, SEAICRN agreed to support Dr. Tan Le Van's "Viral Metagenomics Analysis of Undiagnosed Sepsis Patients Enrolled in SEA-050" protocol. The SEA050 substudy aims to identify known and unknown viral pathogens in undiagnosed patients enrolled in the SEA050 Sepsis Study. This study will not conclude prior to the end of this study period.

As Network support is ending, Leidos Biomed procured the laboratory supplies for this study and shipped the supplies to Dr. Tan's facility at the Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam.

Mexican Emerging Infectious Disease Clinical Research Network (La Red) – Network Coordinating Center

In March 2009, a new influenza virus caused an increase in reports of influenza-like illnesses (ILIs) in North America. By April 2009, the Mexican Ministry of Health (MOH) responded to the public health threat by implementing a series of non-pharmaceutical interventions, which have been widely credited with halting the first wave of the outbreak in Mexico. In September 2009, the Mexican MOH and NIAID signed a letter of intent to develop a coordinated international effort to conduct clinical research on influenza and other respiratory diseases.

The Network Coordinating Center (NCC) assists The Mexican Emerging Infectious Disease Clinical Research Network (La Red) in conducting the highest-quality research in support of multiple protocols. NCC has established standard research procedures as directed by the Network Steering Committee, provides training on these procedures, and ensures the clinical research sites are in accord with these shared procedures; it also supports all other operational and administrative functions to maintain the Network.

The La Red Network conducted the following clinical studies in Mexico, which are being administrated at seven clinical study sites:

- **ILI 002** is an observational study to characterize adults and children with influenza-like illnesses. This study concluded in FY2015, though manuscript preparation continued into FY2016.
- **ILI 2014** is an observational study with the goal to determine the causes of and identify increases in influenza-like illnesses.
- **NTZ-SARI** is a study to evaluate the safety, effectiveness, and tolerability of nitazoxanide (NTZ), in combination with standard of care treating severe acute respiratory illness (SARI) in hospitalized patients.
- **IRC 003** is a study to evaluate whether combination therapy with three antivirals (compared to the standard, one antiviral) will help symptoms resolve faster and with fewer complications.
- **Zik01** is a new study to evaluate potential Zika, chikungunya, and dengue virus infections in Mexico.
- **CN-SARI** is intended to evaluate culture-negative severe ILIs.
- **SPRINT-SARI** (2015–2016 influenza season) is an international observation five-day study of hospitalized ILI. Pending Mexican Central IRB approval, the study will also be conducted during the 2016–2017 influenza season.

During FY2016, the CMRP clinical and associate clinical project managers were involved in the overall administrative management of the research subcontracts; participated in regular status calls with the subcontractor and government customer; reviewed and approved monthly progress reports and invoices, subcontractor travel requests, and travel reports; and monitored the budget and end-of-year budget estimates.

Per a change in Leidos Biomed's prime contract with NCI, research subcontracts and agreements awarded under the OTS contract were modified and re-awarded under the new IDIQ contract (La Red is under TO 15 of the IDIQ). CMRP staff worked diligently to re-award subcontracts with multiple vendors to ensure there were no delays in the work performed in support of the aforementioned clinical trials.

CMRP maintains a research subcontract with SSS to coordinate services for the La Red NCC. The subcontractor provided oversight and management of NCC staff who assisted clinical study sites with the acquisition of materials and supplies, specimen courier services, travel coordination, data management, technical support, meeting coordination, annual reports, invoice processing, and equipment ordering.

The CMRP medical affairs scientist provides ongoing technical leadership to the projects, serves as project lead, meets with principal investigators, revises protocols, oversees several research subcontracts, and provides scientific guidance related to study procedures and subject enrollment, inclusion, and exclusion criteria.

Influenza-Like Illnesses

An influenza-like illness study concept was developed in FY2014 to replace ILI 002 (officially closed in September FY2015). During FY2015, the ILI 2014 study began enrollment. Currently, 6,500-plus participants have been enrolled.

In FY2015, planning began about the development of ILI 2014 sub-studies for potential implementation in FY2016. If other mechanisms of ILI surveillance can be established by the Mexican MOH, and can serve as the basis for the sub-studies, then it is anticipated that ILI 2014 will end in FY2016.

One pilot sub-study, CN-SARI, was planned as a stand-alone clinical trial to identify the etiologic agents associated with severe acute respiratory infection in intubated adult subjects with negative multiplex real-time Polymerase Chain Reaction and microbiology results with 100 subjects enrolled. The timeline for this trial was estimated to be nine months from initiation to study close-out, with TO requirements to develop additional ILI studies after completion of the CN-SARI study.

During FY2016, Leidos Biomed executed a research subcontract with Westat to provide support to the CN-SARI study. Westat worked closely with the La Red NCC and clinical sites to ensure compliance with regulatory requirements, policies, and procedures applying to the conduct of clinical trials research involving human subjects; provide training to site staff; and provide operational assistance to the clinical sites for the preparation and management of studies, including guidance on study-specific questions, preparing procedure and other study-related documentation, and assisted with developing, validating, and implementing data management and sharing plans.

However, due to reprioritization, resources within the La Red Network are primarily focusing efforts towards developing and starting a protocol to evaluate Zika infections in Southern Mexico. As a result, the CN-SARI project was not implemented as originally planned.

Evaluating the Safety, Effectiveness, and Tolerability of Nitazoxanide in Addition to Standard Care for the Treatment of Severe Acute Respiratory Illness in People Who Are Hospitalized (NTZ-SARI)

During FY2016, CMRP staff provided management and oversight to NIAID DCR's respiratory viruses research in the form of technical and scientific leadership, project/procurement and logistics management, subcontract administration, as well as regulatory, safety, and clinical trials monitoring services.

The NTZ-SARI study, "Evaluating the Safety, Effectiveness, and Tolerability of Nitazoxanide in Addition to Standard Care for the Treatment of Severe Acute Respiratory Illness in People Who Are Hospitalized," is expected to screen 500 subjects and randomize 290 subjects who are being hospitalized with severe acute respiratory illness (SARI). Subjects are randomized to treatment with standard of care, or standard of care plus nitazoxanide (NTZ), a compound with broad antiviral properties. The study is conducted in its entirety in the La Red Network in Mexico.

CMRP strategically used several mechanisms to provide clinical trials management services for this initiative. CMRP's research subcontract with a qualified clinical research organization (CRO) allows for the provision of the following: clinical trials management and operational support; protocol implementation and staff training at six clinical sites; administrative and programmatic support; laboratory and specimen collection kit manufacturing, management, and distribution; data management; and statistical support. CMRP also has an established project agreement for the storage and distribution of investigational study product in Mexico City, Mexico.

During the reporting period, a total of six sites were open to enrollment for the NTZ-SARI study. One of the six sites is a new site in Oaxaca; it began enrolling patients in December 2015. To date, 185 subjects have been enrolled.

Enrollment of adults into the NTZ-SARI study paused in FY2016 due to the expiration of the old lot of study agent and delays in receiving the new lot from the manufacturer. CMRP staff worked with the manufacturer to expedite the drug shipment and documentation distribution. The NCC and the CMRP team reviewed the documentation to ensure there would be no problems receiving the kits and getting them through the import process in Mexico. During this review, CMRP became aware of an issue with how the depot was assigning part numbers to the drug kits and negotiated with the depot to amend their process in order to allow the study agent kit's part numbering to remain consistent. When the adult formulation kits were received at the sites, they noted that the kits were numbered with the same numbers as the previous lot. Solutions were discussed with SSS, CMRP, NCC, and the sites in Mexico at a January meeting, and SSS sent a memo to NCC and clinical sites stating that the kit lot number would now be required to be tracked on the Study Agent Accountability Log. An updated version of the revised log (where the kits are tracked when a patient is enrolled) was sent to the sites. The electronic case report forms were updated to require entering the kit lot number as well.

Additionally, the current lot of pediatric formulation for the NTZ-SARI study expired June 30, 2016. A new lot was requested and shipped the first week of June, avoiding the possibility of having to close the study to pediatric enrollments. CMRP facilitated the development and approval of all paperwork prior to this shipment.

Two Data and Safety Monitoring Board (DSMB) reviews were performed in this reporting period. The NIAID DSMB conducted an interim review of the protocol in December 2015; no safety issues were identified and it was recommended that the study continue as planned. The interim efficacy DSMB analysis took place in April 2016 and the DSMB found no deficiencies and recommended that the study continue as planned for full enrollment.

La Red – Zika

The Zika virus is a disease spread to people primarily through the bite of an infected mosquito of the Aedes species; this species is also known to spread dengue and chikungunya viruses. The illness causes mild symptoms such as low fever, rash, and joint pain, and an infection during pregnancy can cause serious birth defects (e.g., microcephaly), as well as other neurological disorders.

The La Red Network is a multi-site collaboration between NIAID and the Mexico MOH that began in September 2009 to conduct clinically relevant studies on emerging infectious diseases. The focus has been on influenza and other respiratory viruses. However, with the emergence of Zika in southern Mexico, La Red developed an observational study, "Evaluation of Potential Zika, Chikungunya, and Dengue Infections in Mexico" (Zik01), to evaluate the cause of Zika-like illnesses in southern Mexico to determine if these illnesses are from Zika, chikungunya, dengue, or other viruses. The CMRP medical affairs scientist is the project leader for NIAID DCR for La Red activities and helped to write the Zik01 protocol in conjunction with Mexico MOH staff.

To support Zik01, the La Red TO was expanded to allow increased capacity for the addition of up to six new sites as well as the addition of support staff at the Network Coordinating Center (NCC). Leidos Biomed established a research subcontract with Westat in May 2016 to provide technical, data management, and clinical trial support services for the Zik01 study. The quick establishment of the subcontract with Westat was imperative to ensure proper and timely preparations were underway for the database, case report forms, and other associated documentation prior to the study start date. Westat leveraged the La Red Network and its infrastructure to provide data management, operational, and manuscript support to the Zik01 study.

In May 2016, the study was approved by the Mexican Central Institutional Review Board and reviewed by COFEPRIS (Mexico's equivalent of the FDA). The La Red NCC expanded to 12 sites total, and hired and trained new staff (e.g., Good Clinical Practices and study-specific trainings). NCC will provide oversite and management of the sites, monitoring support, manuscript preparation assistance, and coordination for basic research use of the study specimens.

For the Zik01 study start-up, supplies and equipment were acquired through the La Red NCC and distributed to the sites. The La Red NCC also conducted site visits to ensure compliance and capacity to conduct clinical research. After protocol training was conducted in Tapachula, Mexico, the study officially began the first week of June 2016.

CMRP initiated efforts to repurpose six laptops, three freezers, and one printer previously used for IRC studies that have closed. Repurposing this equipment is a potential cost savings of approximately $50,000 ($40,500 for freezers, $500 for the printer, and $9,600 for laptops).

Genomic Studies

A NIAID YT at the end of FY2013 requested CMRP's support in completing a genomic study using samples previously collected and stored from subjects under NIAID's Phidisa Project Ia—a prospective, cohort study of HIV infection (both treated and untreated) and risk-related co-infections in the South African National Defence Force. The goals are to identify novel variants in a population of individuals of South African ancestry and host genetic factors associated with: (1) susceptibility or resistance to infection by HIV, hepatitis B (HBV), hepatitis C, gonorrhea, syphilis, and many other diagnosed active infections; (2) disease progression rates; (3) various clinical parameters (e.g., blood cell counts, viral load, liver function tests, lipids, glucose); (4) therapeutic responses; and (5) virus genotype or phenotype. In FY2016, this non-severable YT was amended to support any appropriate HIV genomic study in international settings beyond South Africa. It is likely a genomic study will be included as part of the HIV West Africa project.

The initial work on the Phidisa genomic study has not yet come to fruition due to the political climate in South Africa, but Leidos Biomed has continued to discuss various options for future genomic studies with NIAID DCR as the YT allows for additional genomics studies to be supported.

Once a study commences, CMRP will: (1) provide semiannual status reports detailing research activities and progress; (2) provide programmatic updates related to milestones achieved under studies, research activities, and budget status; (3) provide data reporting and analysis; and (4) manage procurement activities such as purchase orders/subcontract agreements and associated vendor agreements.

CMRP managed and closed out the purchase order agreement with Advanced BioMedical Laboratories after the organization completed DNA extraction on 2,520 samples collected from the INSIGHT network's START study in October 2015. Genomics testing on these samples and on additional DNA extracted samples collected under other studies is anticipated to be performed and will likely support the genome-wide association study (GWAS). Acquisition planning to support genomics testing was initiated during FY2016.

Recombinant Human Interleukin-15

CMRP continued to provide support to the Recombinant Human Interleukin-15 (IL-15) project though oversight and coordination of research subcontractors (Biologics Consulting Group) and Smithers Avanza Laboratories (formerly Avanza Laboratories) to perform pharmacodynamic and pharmacokinetic studies. CMRP also provided technical and management support for a new research subcontract for additional research and development with Georgetown University on the IL-15 cytokine–based therapy in combination with the blockade of immunomodulatory receptors to enhance HIV-specific CD8 T-cell responses to enhanced viral control/ elimination and achieve function cure in HIV-infected patients.

The proposed SOW included two study aims: (1) involve the blockage of the PD1/PD-L1 pathway that will enhance HIV-specific CD8 T-cell responses, and (2) involve in vivo effect of IL-15 and PD-L1 blockage in controlling simian immunodeficiency virus (SIV) responses. It is anticipated that the aim will be completed by September 2016.

Since October 2015, the CMRP technical project manager (TPM) and the assistant TPM have overseen new research and development Study 2078-13161 (Non-GLP Evaluation of SIV-specific response and virology control by treatment combination of IL-15 and Anti-PD-L1 in 12 rhesus monkeys). Study 2078-13161 was completed; it included inoculation of nonhuman primates (NHPs) with SIV, 24 doses of Anti-PDL1, two 10-day cycles of IL-15, and a recovery period for NHPs while data analysis was completed. In addition, leukocyte differentials, SIV-specific immune function analysis, and SIV viral load assessments were completed. The CMRP TPMs worked closely with the Leidos Biomed Subcontracts department, Applied and Developmental Research Directorate (ADRD), and the NIAID DCR government counterparts to manage the execution of subcontract award with Georgetown University in support of ongoing research related to IL-15.

The CMRP TPM shared information and provided expertise and knowledge for the research and development, clinical manufacturing, and animal studies that were carried out under the IL-15 project to support the research and development efforts relating to the use of other cytokines and antibodies for the NIAID-DCR clinical HIV trials. The knowledge and information gained from the oversight of the IL-15 project served as the primary building blocks for developing preliminary project management documents such as cost estimates and statements of work in support of the NIAID DCR's new research and development projects.

Research and Development Initiatives

Leidos Biomed is supporting NIAID DCR's research and development initiatives, which align with DCR's mission to provide multidisciplinary trans-NIAID services, facilitating clinical research and managing special projects as directed by NIAID leadership. DCR's preliminary approach to this effort was the launch of an initial study involving recombinant interleukin-27 (IL-27), a heterodimeric cytokine, which preferentially inhibits HIV-1 replication in HIV-infected monocyte-derived macrophages (MDMs), one of the suspected reservoirs for HIV infection; it also induces HIV resistance in MDMs. Studies of similar cytokines are anticipated, as promising scientific findings have been revealed in this initial study. IL-27 significantly induces interferon-related antiviral genes in MDMs and has been shown to be capable of inhibiting SIV infection in NHP MDMs.

Laboratory work conducted by the ADRD began in FY2014 and support continues to: (1) provide analysis of IL-27 or similar cytokines to be measured in thousands of HIV-infected patients and healthy controls as part of the ongoing evaluation of the utility of IL-27 or similar cytokines including Interleukin-15; and (2) conduct a variety of cell cycle and cell surface marker assays to study the mechanism by which IL-27 inhibits HIV-1 replication in T cells, MDMs, and dendritic cells in vitro.

In December 2015, CMRP and ADRD supported a request for information (RFI) to identify potential subcontractors that have innovative solutions to the examination of IL-27 for inhibition of HIV replication both in vitro and in vivo. In May 2016, Georgetown University was awarded a research subcontract to study the role of IL-27 in viral immunity and its impact as a potential cytokine-based therapy in patients with HIV infection.

The project team has leveraged CMRP's past knowledge, experience, and internal resources from the preclinical development of IL-15, a cytokine also being studied for HIV treatment, for the evaluation of IL-27. This approach has provided the government customer with valuable insight and allowed for more informed decisions when considering the long-term vision of the overall research and development initiative. Laboratory and subcontracting support is expected to continue through 2018.

Indonesia Ministry of Health

NIAID DCR supports clinical research to control and prevent diseases caused by virtually all infectious agents, conducting basic and applied research to develop and evaluate therapeutics, vaccines, and diagnostics.

NIAID's DCR, the National Institute of Health Research and Development (NIHRD) Ministry of Health (MOH) in Indonesia, and a number of Indonesian research sites formed the Indonesia Research Partnership on Infectious Diseases (INA-RESPOND), with the purpose of bringing together clinical and academic medical institutions to develop a robust collaborative infectious disease research network. The aim of the INA-RESPOND Network is to conduct basic and clinical research, increase the understanding of the pathogenesis of diseases, and prevent and treat infectious diseases based on the concerns of the country and in alignment with the priorities of the Indonesian MOH. This beneficial collaboration allows both countries to partner in research and study infectious diseases that affect Indonesia, the surrounding region, and the global community.

NIAID is committed to conducting research necessary to successfully end the fight against HIV/AIDS. The overall prevalence of HIV in Indonesia is estimated to be less than 1 percent; however, there are provinces where the prevalence is

as high as 2.5 percent. The Indonesian MOH is concerned about the steady incidence rate in the country, which is at about 21,000 new cases per year based on reported data. To target this, the Indonesian MOH is in the concept development stage to begin a test-and-treat HIV research initiative in approximately eight of the country's 33 provinces. These eight provinces will receive testing for approximately 1.2–1.8 million individuals in high-risk groups (e.g., pregnant women, prisoners, homosexuals). NIAID DCR will provide support to this research effort for a subset of sites across the various provinces.

NIAID DCR requested that CMRP provide project management and oversight for the completion of at least one HIV study that assesses the impact of testing for and treating HIV in Indonesia. CMRP understands the scope to include the evaluation of two approaches to HIV treatment and prevention in Indonesia. One approach, as noted above, will be implemented in eight provinces by the MOH and then compared to a second approach that will be implemented in other selected provinces that follow the current standard of HIV care and prevention. The intent is to help in the evaluation of a strategy to curb the incidence of HIV/AIDS and curtail the course of what could become a highly prevalent disease. To facilitate the completion of this work scope, CMRP is providing programmatic oversight and managing subcontracts with qualified vendors to provide the necessary support on the ground.

During FY2016, CMRP oversaw activities conducted by SSS, PT Prodia Laboratories (Prodia), and PT Ganesha Aggies Jaya (Ganesha) in support of the following clinical trials:

- AFIRE (INA101) – The Etiology of Acute Febrile Illness Requiring Hospitalization.
- TRIPOD-TB (INA102) – Tuberculosis (TB) Research of INA-RESPOND on Drug Resistant TB.

Prodia, Ganesha, and SSS provided the following support:

- Prodia – Operational and clinical trials support.
- Ganesha – Administrative support for payroll and recruitment activities.
- SSS – Data management and IT support.

The Network continued preparation for the Proactive HIV Study, a prospective observational cohort study of HIV infection and risk-related coinfections/comorbidities in Indonesia. During FY2016, key individuals met to determine the study design and began drafting the protocol.

In FY2015, CMRP participated alongside NIAID DCR and the NSC chair in re-strategizing the infrastructure supporting the INA-RESPOND Network. The purpose of restructuring the Network was to figure out the path that would bring the Network to the next level of independence, i.e., foster engagement to promote self-governance and build in-country capacity. As part of the new infrastructure, CMRP proposed the use of a staffing agency to hire the current and future INA-RESPOND Secretariat staff to help reduce duplication and duality in the Network's management and direction. To further build capacity in-country, the project management team also sought the possibility of having an Indonesian-based vendor support the Network as opposed to a foreign-based vendor. The advantages of seeking a local vendor would include a company that is familiar with the local culture, therefore would better understand the issues arising in different situations and would be available real-time to address the needs of the expanding Network (no time zone difference). Based on these concepts, Leidos Biomed established the following research subcontracts and blanket purchasing agreement during FY2016:

- Prodia – CMRP worked with the Subcontracts Department to fully execute the agreement with Prodia on February 2, 2016. During FY2016, Prodia established agreements with eight of the nine study sites. Prodia also provided logistical and financial support for the Network (i.e., purchased needed equipment and monitored the budget). In order to realign the management of costs incurred by the Network, the subcontract was modified in July 2016 and a new research subcontract was executed with Prodia on June 6, 2016. As a result of the modification, HIV/TB-related costs generated by the Network were easily separated and routed to the appropriate HIV/TB-funded project ID. The newly established agreement supported non-HIV/TB activities, which were allocated to the appropriate biodefense funded project ID.
- Ganesha – CMRP worked with the Subcontracts Department to fully execute an agreement with Prodia on December 7, 2015. During FY2016, Ganesha hired two staff members to support laboratory activities.
- SSS – On April 28, 2016, CMRP worked with the Subcontracts Department to establish a temporary agreement with SSS to support the transition of data management and IT activities to other appropriate parties, as determined by NIAID DCR, CMRP, and the Network committee chair. During FY2016, CMRP ensured the effective collaboration between SSS and NIAID Office of Cyber Infrastructure and Computational Biology (OCICB) to complete transition activities.

The establishment of two agreements with Indonesian vendors (Ganesha and Prodia) provides long-term cost-savings, as labor and other general costs will be incurred per acceptable Indonesian standards and in the local currency, which is favorable to the project at this time.

Historically, the establishment of site agreements with institutions within Indonesia has taken as many as six months. Based on lessons learned, the Secretariat, CMRP, and Prodia worked together to create a version of a contract template and site budget that was concise and would be easily understood by the Institution's Legal or Contracts Department (depending on the study site). By June 2016, eight out of the nine Institutions participating in the Network had executed their site agreement and the ninth agreement was executed in late FY2016.

During FY2016, the establishment of the implementation agreement continued to be finalized between the Indonesian government and the U.S. Department of State. While the implementation agreement is being finalized, the project team continues to strategize on future work; however, the lack of an implementation agreement inhibits the ability to continue study efforts related to TB and HIV research. Activities related to the TB study and upcoming HIV study have begun, as feasible and allowable; the TB study was initiated at three study sites and was put on hold in December 2015 due to ongoing political issues. During FY2016, the addition of an overarching health agreement became a requirement for health-related collaborations and initiatives between the U.S. and Indonesian governments. The establishment of this agreement is currently being discussed between both governments.

As the Secretariat staff transitioned into being more independent from a functional standpoint, CMRP worked with SSS for the Secretariat staff to obtain "@ina-respond.net" email addresses to support identifying them as Network staff. CMRP also supported the procurement of computer software and equipment to replace licenses previously owned by SSS and other items that were no longer functional.

In order to ensure Prodia's knowledge of U.S. regulations, Leidos Biomed's Subcontracts Department provided training to the team in January 2016.

During FY2016, significant progress was made by the clinical operations team to finalize the INA-RESPOND standard operating procedures (SOPs), guidance documents, and additional supporting documentation. Included are general, regulatory, and clinical monitoring processes for the Network. Work also began on the development of the SOPs and guidance documents for data management and information technology.

At the request of the NSC Chair, and supported by NIAID DCR, CMRP and the Secretariat Finance Manager worked collaboratively to establish an Honorarium and Travel Guideline. This guideline was created to clarify the process for the provision of honoraria and business travel arrangements for Indonesian travelers and meeting participants that are more in line with Indonesian standards. With regards to travel, the guideline outlines new Meals and Incidental Expenses (M&IE) and lodging rates for domestic travel that are less than the U.S. rates, resulting in cost savings to the project.

CMRP and NIAID DCR personnel rotate travels to Indonesia in order to cut costs while providing coverage on the ground, as opposed to all team members traveling at the same time.

During FY2016, the clinical project managers reviewed invoices with Ganesha to determine cost savings incurred. Per the agreement with Ganesha, invoices that are processed within 20 days of submission to Leidos Biomed (Net 20) receive a 2 percent discount of the invoiced amount.

HIV Replication Study

Leidos Biomed continues to support the technical, scientific, and program/project oversight for the HIV Replication Study. The goal of this study is to look for HIV replication in privileged compartments (e.g., brain) that cannot be safely studied in living individuals. The study will investigate up to 50 subjects, both pediatric and adult, who had suppressed HIV/AIDS and died of other causes.

CMRP collaborated with the Biospecimen Research Group (BRG) to consider vendors that provided the deceased donor specimens for the GTEx (Genotype-Tissue Expression) Project. CMRP provides programmatic oversight for the research subcontract(s) and collaborates with the ADRD, which conducts the analysis of the specimens.

Given the similar nature of the HIV Replication Study to the GTEx work, the technical project manager continues to collaborate with BRG to learn more about the vendors subcontracted for the GTEx tissue collection. This collaboration is resulting in a streamlined approach to reaching out to the potential vendors and for getting the agreements in place. Plans are to collect approximately 20 tissue samples per subject from the following example compartments: lymph node, spleen, GI tract, brain, tonsil, heart, liver, pancreas, kidney, and blood pool. All subjects will have HIV confirmatory testing and subsequent tissue samples will be shipped to the ADRD laboratory for DNA PCR testing; RNA PCR testing will be conducted on a subset of samples. As the project evolves, the need for other laboratory tests could be identified. This support is expected to continue through 2018.

Mali Clinical Research Program

With the overarching goals to develop sustainable research programs in geographic areas of high infectious disease burden and enhance the capacity of research sites throughout Africa to perform clinical research in accordance with International Conference on Harmonization/Good Clinical Practices (ICH/GCP) guidelines and

applicable U.S. government–mandated regulatory requirements, NIAID DCR planned a partnership initiative and requested CMRP to facilitate and manage the program.

Specifically, CMRP was asked to provide support for building the clinical research infrastructure necessary in Mali to carry out the Strategic Timing of Antiretroviral Treatment (START) study, research the pathogenesis of TB and its intersection with HIV, and study emerging infectious diseases with hemorrhagic viruses in the region. To accomplish this, CMRP is providing management and oversight for the establishment of the University Clinical Research Center (UCRC) infrastructure and resources, as well as strengthening research capacity for ongoing University of Sciences, Techniques and Technologies of Bamako (USTTB) research platforms. A subcontract with USTTB is serving as one facet to facilitate the development of coordinated clinical research programs in Mali, enhance the existing clinical research program and facilitate growth and sustainability, stimulate clinical research in West Africa that is guided by international standards and principles, and develop an excellent research environment that will attract researchers worldwide and foster collaborations.

Building on the momentum that began in FY2015, priorities in FY2016 continued to focus on building the UCRC staffing structure and capacity building efforts for the biosafety level 3 lab (BSL-3) team and laboratory staff who fall under the umbrella of the UCRC, planning a renovation of a building identified to house the UCRC operations, and preparing to initiate the first clinical research study managed by UCRC. This study, partially funded by the World Health Organization (WHO) and conducted in Mali, Guinea, and Cote d'Ivoire, aims to quantify the level of serological protection (seroprevalence) against polio virus serotypes 1, 2, and 3 in high-risk areas. This will allow for a better understanding of the risk of possible undetected circulation, emergence of vaccine-derived polio viruses, and polio virus outbreaks following importation.

In early FY2016, a team of NIAID DCR and CMRP staff traveled to Mali to work on the recertification of the BSL-3 lab hoods, inventory management, and the project's ongoing administrative and managerial needs. Inventory management is a key component of maintaining the lab supported by UCRC. During the trip, the CMRP special projects administrator (SPA) participated in a major overhaul of the main supply and equipment storage areas. This process involved a physical reorganization of all supply areas within the labs and also the external storage areas to increase space availability by approximately 30 percent and improve inventory identification efficiencies.

During the same trip, members of the lab, the CMRP clinical project manager (CPM), and the Mali Service Center (MSC) staff met with the local lab supply vendors to discuss the concerns that each group has experienced and to determine how relations could be improved. The conclusion of this meeting led to the development of a fully detailed procurement plan with SOPs, a communication plan, standard procurement documents, and inventory and logistics tracking tools.

In October 2015, the second UCRC Governing Board (GB) meeting was held on the main campus at NIH in Bethesda, MD. During this meeting, the GB endorsed the pending items on the GB Terms of Reference, discussed outstanding items from the previous GB meeting, endorsed the architect rendering for the renovation proposed for the UCRC site, and endorsed the WHO polio seroprevalence study.

In November 2015, Mali's Radisson Hotel located in Bamako was attacked by terrorists. This devastating situation was a major setback to the project as travel plans for an Executive Committee meeting were halted, the agenda was abbreviated, and the meeting was held via web conferencing with the UCRC team members and MOH in Mali. The inability to travel to Mali and conduct meetings face-to-face, along with the general security issues in the country, created circumstances that momentarily slowed progress on the building renovation, the hiring of UCRC staff, and the momentum of the project that was starting to take place.

CMRP staff was subsequently allowed to travel under strict guidelines and provide on-the-ground support to the UCRC operations. The trips went very well and a significant amount of work was accomplished, including selection of the general contractor for the UCRC building renovation, planning for the polio study, and discussions of new collaborations. The travel is continuously being monitored and staff members were permitted to travel in July and August. Despite the travel concerns, during the course of the year, seven trips were taken by the CMRP Mali project team members to Bamako, Mali, to provide on-the-ground support to the UCRC operations.

Ongoing capacity building efforts have included the launch of the UCRC SharePoint site to serve as a collaborative platform for all UCRC and affiliated members to work and manage project-related documents. The CMRP SPA developed the SharePoint site from scratch, worked with the team to identify the folders and site layout, and set up accounts for each staff member. A multi-phase pilot testing between members of UCRC and Leidos Biomed was done to confirm content and usability of the SharePoint site. An SOP and training were developed to ensure an understanding and ease of use, including management of the site. The SharePoint was officially rolled out in June 2016.

NIAID DCR and CMRP worked with the UCRC and MSC members to plan the roll-out of this study. This involved ensuring that Mali, Guinea, Cote d'Ivoire, and WHO submitted the protocol to their respective IRBs to avoid delays in study initiation. A fair amount of time was spent reviewing the procurement needs and coordinating the shipment of necessary supplies and equipment. The CMRP administrative assistant ensured what items and quantities needed to be ordered for each site, and prepared pricing information. The study began first in Mali in mid-June, and in Guinea and Cote d'Ivoire thereafter. Efforts are being coordinated to send the results to WHO. The UCRC team and EC members continue to research and review proposals of potential protocols for studies that may be conducted by the UCRC in the near future.

In December, Leidos Biomed quickly hired a medical affairs scientist with the appropriate subject matter expertise to support the project's clinical research needs. This new team member facilitates the implementation of UCRC's scientific activities; timely protocol development, implementation, and analysis; and publications.

Through the USTTB subcontract, several positions were hired to support the UCRC operations. A Fellow started in February and is developing independent and collaborative research themes in the field of cellular and molecular immunology of infectious diseases, providing scientific expertise, guidance, and support for UCRC research needs. In March, a scientific program manager was hired to manage UCRC protocols, clinical research studies, and UCRC on-going activities. In May, an administrative assistant was hired to support the UCRC director and the UCRC team overall. UCRC continues to build its staff and has plans to hire a scientific training coordinator and a research coordinator in the near future.

CMRP supported planning activities related to the UCRC building site renovation, participating in conference calls, collaborating on the development of a thorough project scope, and reviewing contractor bids. In April 2016, MSC selected a general contractor and renovations began in June; it is anticipated that the work will be completed in early FY2017.

Viral Hemorrhagic Fevers: Ebola

Research supported and conducted by NIAID strives to understand, treat, and ultimately prevent the myriad of infectious, immunologic, and allergic diseases threatening the health of millions of people in the U.S. and around the world. Against a background of established infections, epidemics of new and old infectious diseases periodically emerge. This threat has been increasingly recognized over the last decade. Emerging and re-emerging infectious diseases (ERIDs), such as viral hemorrhagic fevers (VHFs), Ebola hemorrhagic fever, Lassa fever, and Marburg hemorrhagic fever, are of particular concern given the potential for significant morbidity and mortality. This concern fosters the overall goal of a better understanding of the diseases and therapeutic options, and of improving medical outcomes for afflicted patients.

Clinical Research Studies

For NIAID's response to the Ebola virus disease (EVD) initiative, CMRP is managing a portfolio of international clinical research studies that serve as a comprehensive research platform allowing DCR to effectively respond to VHF and other ERIDs through anticipation, early reaction, disease characterization, treatment, collaboration, and flexibility. In FY2016, CMRP continued and expanded efforts on the three studies that were launched in FY2015: Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) I, II, and III. In addition, CMRP managed a number of special projects associated with the PREVAIL program, such as renovations at Maferinyah in Guinea, Project C.U.R.E., developing and establishing an inventory management system, developing a biorepository for the PREVAIL specimens in Liberia and Guinea, developing an imaging sciences center at John F. Kennedy (JFK) Medical Center in Monrovia, Liberia, and developing a protocol visit scheduling tool.

Early 2016 marked the one-year anniversary of PREVAIL I (NIAID Protocol # 15-I-N071), a study of two vaccine candidates. Follow-up visit attendance by participants remained at an incredible 96 percent, largely due to the efforts of the social mobilization team, which includes participant trackers who work in local communities to follow and support participants, address community concerns, and serve as knowledgeable, trusted resources for the public. In May 2016, an amendment was approved to instate long-term follow-up visits for PREVAIL I participants for another four years. Initial results from PREVAIL I, as reported by the principal investigator (PI) at the Conference on Retroviruses and Opportunistic Infections (CROI) in February 2016, indicated that both vaccines stimulated a good immune response in the early weeks after vaccination and were well tolerated, even among people with HIV. In an unexpected twist, the study also found that a number of participants had evidence of a past Ebola virus infection, although none reported a known history of EVD.

In November 2015, a new cluster outbreak of three Ebola cases occurred in Liberia two months after the country had been declared Ebola-free. Swift action from all partners led to the development, approval, and implementation of an amendment for PREVAIL I, allowing for cluster vaccinations of close contacts from the Ebola cases. Vaccinations

occurred daily from November 23 through December 11, with a total of 210 volunteers enrolled (close contacts and health care workers). Another cluster of cases occurred in Guinea and Liberia in March and April, respectively. ZMapp, the triple monoclonal antibody agent used in PREVAIL II, was transferred to other agencies to administer in Guinea and Liberia. In addition, vaccinations were transferred to the MOH in Liberia. CMRP provided response assistance with on-the-ground support from trained personnel, and the social mobilization team had an active role in recruiting and tracking people for the vaccinations.

The final participants were enrolled in PREVAIL II (Medical Counter-Measures Randomized Clinical Trial, NIH Protocol # 15-I-0083) by early FY2016, reaching a total enrollment of 72 participants from Guinea, Sierra Leone, Liberia, and the U.S. PREVAIL II officially closed its sites to enrollment in January 2016; data was unblinded soon after. Long-term follow-up continued in Sierra Leone through the end of FY2016. As reported by the PI at the CROI meetings in February 2016, an initial look at results from PREVAIL II—the only clinical trial evaluating whether the experimental Ebola therapy known as ZMapp is safe and effective—revealed that the treatment is well tolerated and suggested it may hold some promise as an Ebola treatment. However, the study could not enroll enough patients to prove that ZMapp offers significantly more healing benefits than supportive care alone.

Enrollment of Ebola survivors and close contacts in the Ebola Natural History Study (PREVAIL III, NIAID Protocol # 15-I-N122) grew to more than 3,600 participants (over 1,090 survivors and over 2,380 close contacts) by the end of August 2016. CMRP continued its support of this study by providing regulatory oversight and protocol-specific technical support; offering logistical procurement, shipping, meeting, and travel support; awarding research subcontracts; providing on-site training; and providing overall project management.

As reported by the PI at the CROI meeting in February 2016, eye complications, muscle and joint troubles, and neurologic issues topped the list of medical problems diagnosed more than a year after recovery from EVD. The clinical picture emerging from PREVAIL III shows that survivors and their contacts suffer from many health problems.

PREVAIL III increased its scope through the initiation and expansion of several sub-studies. Three sub-studies began that collected and examined bodily fluids of survivors (semen, breast milk, and vaginal fluids). Expectant female survivors and partners of survivors started enrollment in the birth cohort sub-study, which follows outcomes of their pregnancies and the health of the neonates after birth. The existing neurology sub-study was expanded to include a pediatric cohort and collection of lumbar punctures, and enrollment in the ophthalmology sub-study continued and expanded to all PREVAIL III participants. CMRP supported the sub-study operations through several efforts, including repurposing existing rooms for sub-study collections or renovating new facilities to accommodate expansion efforts; establishing standard operating procedures; providing regulatory oversight; offering logistical procurement, shipping, meeting, and travel support; awarding research subcontracts; providing on-site training; and providing overall project management.

Three new studies (PREVAIL IV, Partnership for Research on Ebola Vaccines [PREVAC], and PREPARE, a multicenter study of the immunogenicity of recombinant vesicular stomatitis vaccine for Ebola-Zaire [rVSVΔG-ZEBOV GP] for pre-exposure prophylaxis in individuals at occupational risk for Ebola virus exposure) are in various stages of development and/or have been initiated and supported by CMRP resources.

PREVAIL IV was rapidly initiated due to concerns of ongoing EVD transmission in Liberia. Launching PREVAIL IV in a short time frame involved a broad CMRP team effort to meet customer expectations. In addition to the routine regulatory and clinical trials management services, the project management support services were spread across multiple CMRP staff members to facilitate logistical planning for study initiation. CMRP used an "all-hands-on-deck" approach and garnered resources from various groups to efficiently and effectively manage study objectives and timelines.

Screening for the PREVAIL IV study (Double-Blind, Randomized, Two-Phase, Placebo-Controlled Phase II Trial of GS 5734 to Assess the Antiviral Activity, Longer-Term Clearance of Ebola Virus, and Safety in Ebola Survivors with Evidence of Ebola Virus Persistence, Protocol # 16-I-N137) began at the end of June 2016, and infrastructures related to conducting the study were established. These efforts included designing the protocol and related study documents; providing regulatory and DSMB oversight; offering logistical shipping, meeting, and travel support; awarding research subcontracts; determining efficient procurement mechanisms; procuring supplies for in-country operations; providing on-site training; establishing pharmacy and laboratory operations; and providing overall project management. Site laboratory electrical systems were also renovated to ensure sustainability and continuous operations during the study. Members of CMRP's Regulatory Affairs Group, Clinical Trials Management Team, Clinical Safety Office, and Protocol Navigation Program worked quickly to ensure the protocol would be cleared/approved to proceed, allowing initiation of the study in June 2016. Plans are to enroll 60 male participants by November 2016; 19 were enrolled as of August 2016.

The (PREVAC study, a multi-country study, is planned for initiation in Liberia, Guinea, and Sierra Leone by late FY2016. This study is a placebo-controlled, randomized, double-blind trial in adults and children. Infrastructures related to the study conduct were established in conjunction with other sponsors. CMRP shared successful methodologies learned from establishing PREVAIL I–IV with partners to initiate and conduct the study. CMRP leveraged the experience of local staff and sites experienced with PREVAIL protocols to support multiple efforts, including designing methodologies associated with patient recruitment, follow-up of subjects, and data management; implementing biometric measures to ensure patient safety and study integrity; providing regulatory oversight; offering logistical shipping, meeting, and travel support; awarding research subcontracts; determining efficient procurement mechanisms; providing on-site training; establishing pharmacy, laboratory, and repository operations; providing overall project management; and facilitating multi-national communications and translations. CMRP also initiated and managed significant renovations and repairs for a study site in Maférinyah, Guinea, in preparation for PREVAC and to develop long-term research capacity.

The third study developed and IRB approved during FY2016 was PREPARE, Protocol # 16-I-0053. PREPARE is planned as a U.S.-based, multi-center study to evaluate the durability of the immune response following the administration of the investigational rVSVΔG-ZEBOV GP vaccine (V920) as pre-exposure prophylaxis for up to 1,000 adults who have an occupational risk for potential exposure to the Ebola virus. It is anticipated that the study will be initiated near the end of FY2016.

The CMRP Ebola support team worked to address several challenges associated with these complex clinical research efforts. A brief summary of the overarching challenges and solutions is outlined below.

- *Lack of site infrastructure/in-country resources*: In Liberia, the bulk of study site renovations were completed prior to FY2016, although repairs and renovations continued as needed. Several renovations were also made to the Liberia Institute for Biomedical Research (LIBR) laboratory facilities and the PREVAC study site in Maférinyah, Guinea. However, lack of in-country resources continued to be a challenge. For example, in early January 2016, a series of power surges occurred at the main JFK Hospital facility in Monrovia that disrupted PREVAIL operations and prevented the use of the laboratory until repairs could be made. In order to ensure high-quality work and prevent further issues, the electrical system was thoroughly investigated by independent experts, daily routine inspections were formalized, and repairs were made with close quality control oversight. Furthermore, the availability of sufficient Internet connectivity for clinical operations continued to be a challenge. CMRP IT personnel worked closely with the study sites to install improved networking solutions and enhance cross-functional communications.
- *High visibility*: The high-profile nature of the Ebola response effort puts the study under an extra degree of scrutiny that layers an additional level of pressure on staff members. Key staff members continue to provide project management and oversight on all aspects of the project. The medical affairs scientists are helping with community outreach and social mobilization to ensure community support and encourage study recruitment/enrollment.
- *Unpredictability*: Supporting the study of infectious diseases such as Ebola remains a challenge due to the unpredictable nature of the disease. Continued strategic planning and open communication among PREVAIL leadership are critical. Following the re-emergence of Ebola in late November 2015, through a series of urgent and open meetings among leadership and staff, CMRP supported the launch of a rapid response to these new outbreaks through an amendment to the PREVAIL I protocol. However, this incident served as a reminder of the unpredictability of the Ebola virus, and underlined the need for open communication and risk management planning at all operational levels. On a daily basis, CMRP staff evaluates and prepares to address risks both foreseen and unforeseen, from ensuring safety and security protocols are in place to addressing the psychosocial concerns of survivors. Furthermore, a manual of operations was drafted in order to rapidly mobilize in-country resources should another outbreak occur. Intensive security training has also been implemented for all regional travelers.

Special Projects

CMRP provided logistical and shipping support for donations of baby food and medical supplies, generously provided by non-government organizations (NGOs) interested in post-crisis relief and rebuilding efforts, to partners in Liberia. This support included arranging for shipment from the U.S. to Liberia; identifying orphanage and hospital recipients and matching recipient needs with donation availability; identifying, clearing, and acquiring sufficient, secure warehouse space; clearing donations through transportation authorities; physically sorting supplies; and transporting supplies to recipients.

In order to manage the inventory required for multiple studies at multiple sites and to support the considerable amount of supplies and materials that are procured and shipped to Liberia for PREVAIL activities, CMRP evaluated and facilitated the development of an inventory management system that could meet the unique requirements of the project. Barcoding equipment has been procured, and a hosting platform has been established and configured with data fields from the current inventory tracker to be evaluated for functionality and usability. A barcoding system for inventory was implemented during this period, thereby reducing the number of man-hours needed to manage supplies and eliminating duplicate orders.

For a PREVAIL III (Ebola Natural History) cohort sub-study, CMRP is assisting with the institution of an imaging sciences center at JFK Medical Center to expand local research and clinical capacity. Neuroimaging capacity is of particular importance to Ebola research, given the interest in neurological sequelae, but has a myriad of other research and clinical applications. CMRP is providing support at all stages in this multi-step process, including consulting with subject matter experts, facilitating site visits for vendors and other stakeholders, procuring equipment suitable for a lower-resource environment, building local human capacity through training and other support, and assessing for long-term renovation solutions that would allow for future expansion of local imaging capacity.

PREVAIL III aims to assess the neurological effects of EVD on pediatric and adult EVD survivors. Imaging will play a key role in evaluating possible neurological manifestations. Currently, there are no imaging capabilities in Liberia; therefore, establishing such imaging modalities is critical to these future endeavors. NIAID DCR requested that Leidos Biomed provide a complete turnkey solution for the procurement, renovation, installation, training, and maintenance of a CT scanner and MR device. Led by the CMRP CCS clinical project manager, a group of subject matter experts (SMEs) was formed to prepare an imaging proposal and requirements document. Leidos Biomed issued the RFP in November to three pre-selected vendors. Responses were received in December, and an SME imaging meeting was held in early January to conduct a review and discussion. After obtaining clarifications, negotiations began in February 2016; the procurement transitioned to the Leidos Biomed Subcontracts department, and two subcontracts were established to support this turnkey imaging solution.

The imaging equipment will be located at JFK Medical Center. A letter of intent to solidify the partnership and understanding of the proposed imaging program between NIAID, the Liberia MOH, and JFK was signed in March 2016. Two working groups between the partnerships are providing input on renovation plans and creating a finance and resource plan. An on-site feasibility assessment was conducted at JFK in May by the renovation subcontractor to provide a complete assessment of the infrastructure, design, costs, timeline, critical dependencies, and recommendations for the identified imaging space. Upon receipt of the resulting assessment report, verification of available budget, and determination that the proposed renovation will create sufficient infrastructure to support all aspects of the imaging space, the renovation will be approved to begin. The complete turnkey solution is expected to take approximately one year for the renovation, installation, and robust training.

Additional software development activities included establishing an electronic scheduling tool to facilitate visit tracking for trial participants and replacing a paper-based process that was inefficient and prone to errors. The project is in the end stages of the evaluation and assessment phase, with latency tests of the network connection being conducted in addition to interface usability by potential end users.

Development of a long-term repository solution to store PREVAIL specimens in Liberia and Guinea is an ongoing special project. Evaluations continued for a PREVAIL specimen repository in Liberia and Guinea, with potential space identified. The team is working with the laboratory leads to determine the total number of specimens for all of the studies in order to calculate the number of freezers required. The framework for a sustainable specimen repository is being developed in collaboration with a number of experts and stakeholders.

Research Subcontract Management

Multiple subcontracts were established to support the clinical research efforts. Seven were awarded to one vendor to provide statistical and trial operational support for each study, including feasibility assessments and data management, as well as expertise to support protocol development and implementation. One was awarded to oversee the administration and disbursement of local resources in Liberia and Guinea (i.e., financial and administrative in-country support). Two were established to help educate, engage, and communicate with the local communities about Ebola and the clinical trials supported by NIAID: one to address the social mobilization needs within the country, including participant follow-up and tracking, and another to support social analytics and biometrics. A subcontract was established for laboratory and repository support to all studies, and another to provide clinical consultancy to the Ebola response team in support of laboratory set-up. Three were awarded for country-specific support: one to manage the conduct of PREVAC in Guinea, a second for the

continuation of long-term support in Sierra Leone for PREVAIL II, and a third to provide cold chain management in Liberia.

Logistical Oversight

Procurement planning, execution, and distribution of multiple classes of supplies, including hazardous materials, perishable freight, and sensitive equipment, are conducted by the logistics team. Inventory, storage, and flow of supplies from port of entry to the appropriate medical facilities has been coordinated by on-the-ground support, including local support staff and the U.S. Embassy for customs clearance, transportation, warehousing, and distribution. Given the volume of procurements and international shipments arranged for these studies (over 2,350 purchase orders on 45 shipments between October 2015 and September 2016), shipping and logistics personnel have arranged shipping more efficiently, assessing shipment priority and space requirements to consolidate shipments and reduce costs and physical waste. Personnel overseeing study sites have also been mindful about supply ordering and shipments, purchasing locally or in bulk where possible, and reducing the need for rush orders by putting in supply requests as early as possible.

Facility Renovations

Many repairs and renovations were necessary at the West African medical facilities that serve as research sites. Emphasis has been placed on the sustainability of these renovations and repairs to reduce the long-term costs and ensure the safety and security of human and material resources. These decisions were carefully researched and evaluated with the aid of local experts, and quality oversight was used for more extensive operations to ensure lasting, quality repairs.

Capacity Building

In March 2016, a strategic planning meeting was held between U.S. and Liberian stakeholders, for which CMRP provided logistical and travel support. A common interest from all stakeholders was sustainability and long-term research capacity building in Liberia, particularly given the unique opportunity to use lessons learned from the Ebola crisis to prepare for future research and public health needs.

CMRP supported several initiatives to encourage and promote regional research opportunities and professional development activities. These initiatives included logistical and travel support for a sub-regional research consortium in May 2016 and scientific writing workshops designed to give authors the opportunities to share unique perspectives on the Ebola crisis and related research challenges. In Liberia, the PREVAIL sites were stocked with textbooks on clinical research to serve as an educational resource for the staff. Neurology and ophthalmology team members have also given educational lectures and presentations for interested hospital personnel. CMRP also supported local staff members who sought further education or certification related to clinical research.

While assisting with building the laboratory and clinic infrastructures in Liberia, CMRP facilitated the training of local Liberian pharmacy, laboratory, and clinical staff to ensure standard procedures for filling syringes, obtaining informed consents, aliquoting and processing samples, vaccinating and monitoring participants during the post-vaccination period, and tracking participants. The Regulatory Compliance and Human Subjects Protection Program (RCHSPP) Regulatory Affairs group provided on-site training in Liberia to PREVAIL operations staff on regulatory guidelines and ethical principles in preparation for PREVAIL IV.

In May 2016, CMRP hosted the visit of two Liberian PREVAIL clinical research site managers to NIH/DCR/NIAID and Leidos Biomed. CMRP personnel met with Liberian site managers to discuss training program best practices. This was the first of several planned meetings with additional personnel from the Liberian PREVAIL operations team.

IT Infrastructure and Technical Support

Within the LIBR, a contract with a local Liberian Internet service provider was established to provide high-speed Internet connectivity for research operations and replace an outdated satellite connection that suffered from high latency and low throughput. Installation of wireless networking equipment, cabling, access points, uninterruptible power supplies, and centralized, web-based remote policy management capabilities were completed, and the local area network was made available for use by staff. Technical staff continue to monitor and optimize the Internet service provider backbone to ensure negotiated bandwidth amounts are being delivered. With the improved Internet connectivity, two independent video conferencing platforms were deployed, providing LIBR clinical staff with real-time video communication capabilities for collaboration and information sharing with NIH staff located in the U.S.

At Redemption Hospital in New Kru Town, one of the initial centers for treating Ebola patients and the only public health care facility in an area serving over 90,000 people, new networking equipment was deployed to improve existing Internet connectivity and provide centralized, web-based policy management and application-aware traffic shaping capabilities for the optimization of available bandwidth. Similarly, at JFK Medical Center, two

independent network backbone lines were established to provide Internet connectivity and video conferencing capabilities to the entire second floor, which is used in support of PREVAIL clinical and operational activities. Remote monitoring, management, and technical support services are provided to ensure optimal performance and bandwidth allocation.

Discussions with Leidos Biomed and government staff have continued regarding potential applications of the biometrics platform to support clinical efforts in West Africa. This technology would be used to identify study participants during enrollment and subsequent follow-up visits, thereby eliminating potential fraud and/or the possibility of study participants receiving more than the expected dose of a particular study agent. Customizations and configuration changes have been made to support the workflow of the clinical research efforts at sites in Liberia. The stakeholders have been engaged and are providing activity direction and guiding the future implementation path.

To provide the PREVAIL Social Mobilization Committee (SMC) with a barometer of sentiment for Ebola-related clinical research activities in West Africa, a social analytics platform was developed with the ability to gather Ebola-related social and traditional media coverage from the Internet, as well as accept input from in-country data collectors with access to local television, radio, and newspapers. The corresponding portal is interactive and allows for deep dives into content, and features a geospatial locater that highlights areas of activity on a map of the West African countries. Weekly reports have been developed and meetings held with the SMC communications pillar to review the content and determine suitable strategies to dispel rumors and direct future communication events.

To facilitate collaboration with PREVAIL staff, partners, and other members involved in the NIAID West Africa research activities, several video conferencing systems have been operationalized in two different locations in Liberia (LIBR and JFK Medical Center). These technologies have bridged a significant communication barrier, as the communications infrastructure in Liberia relies nearly exclusively on cellular technology, and meetings were often held using a single cellular telephone. Introduction of the commercial-grade equipment allowed for various web-based technologies such as Skype and GoToMeeting to be used for meetings and online collaboration, thereby improving communication opportunities with staff in the U.S. as well as reducing the amount of travel necessary to participate in in-person meetings.

Providing information technology (IT) infrastructure and technical support for the West Africa Ebola initiatives created a unique opportunity to build capacity and mentor Liberian IT colleagues. In deploying complete networking solutions and video conferencing platforms to multiple sites, the CMRP IT group was able to expose colleagues to new technologies and provide them with experience and methods in utilizing and maintaining the equipment. Additionally, the CMRP IT group meets routinely with the staff via video conferencing to discuss technical issues and provide guidance on potential solutions.

Travel Coordination and Operational/Logistical Support

A high volume of travel to West Africa continued in FY2016. Numerous travel logistics are associated with each travel request. Upon receipt of a pre-travel authorization form, CMRP's coordination activities include: (1) tracking dates of issuance and expiration for country visa; (2) collaborating with the administrative team and Leidos Biomed Travel Office to prepare travel justifications and coordinate various logistics; (3) preparing travel data for NIAID DCR weekly calls; (4) submitting the required electronic Country Clearance (eCC) for each traveler to Liberia, Guinea, and Sierra Leone; and (5) confirming safe re-entry to the U.S. for all international travelers. CMRP prepared over 400 travel packages and 150 travel requests specifically in support of the Ebola initiative. In addition, CMRP initiated and secured over 20 visas for the West African countries of Liberia, Guinea, and Sierra Leone in support of Ebola-related travel for Leidos Biomed employees and non-employees.

As of October 1, 2015, the U.S. Embassy in Liberia discontinued providing health care for CMRP/Leidos Biomed staff traveling to Liberia. This led to an intensive undertaking by CMRP to identify and secure a medical facility for staff to receive medical attention, as needed, while in Liberia. A formal agreement was reached in January 2016 between Leidos Biomed and Aspen Medical International, allowing CMRP/Leidos Biomed travelers to have access to a medical facility 24 hours a day/7 days a week. Initial six-month subscriptions were purchased and are transferrable among travelers. Weekly lists are managed and sent by CMRP to Aspen Medical International. Leidos Biomed Subcontracts Department released a letter to all subcontractors to make them aware of Aspen Medical International, their ability to engage this or a similar service, and invoice the access fee costs in accordance with their agreement. A non-employee traveler letter is under review to notify non-employees of this service, but any and all insurance expenses will be at their own expense. The Leidos Biomed contract does not allow this as a reimbursable expense for non-employees.

In April 2016, Aspen Medical International agreed to extend the existing agreement to CMRP/ Leidos Biomed staff traveling to Sierra Leone, thus ensuring access 24 hours a day/7 days a week to a medical facility in Sierra Leone. CMRP generates a weekly report for Aspen Medical International to identify who is in-country and dates of travel.

Project Management Policy Implementation

As directed by the CMRP director, the Project Management Team (PMT) assisted the Ebola team with implementation of the FNLCR pilot project management policy that provides a framework for managing collaborative work between FNLCR and NCI. The support activities included the development and update of the Ebola program charter and project management plans for PREVAIL I, II, III, and IV, PREVAC, PREPARE studies, and the HIV Clinical Research Project in West Africa. These documents describe the approach and identify specific methods and procedures, both managerial and technical, to be used for executing and monitoring all Ebola response initiatives. The documents are required program artifacts per Leidos Biomed's project management policy, and will ensure that the clinical and scientific projects are executed in accordance with customer requirements. As living documents, they will be kept up to date throughout the course of the various projects within DCR.

PMT assisted with the operational, logistical, and programmatic support needs to ensure continuity of Leidos Biomed's rapid response capabilities in West Africa. PMT helped the CMRP Ebola team by providing rotating staff for on-the-ground services needed to support operations in Liberia, Sierra Leone, and Guinea. PMT also provided project management support to Project Cure, which aims to receive and donate a large amount of hospital equipment and supplies (in 7 shipments, 40 foot containers) to 8 hospitals (JFK, Redemption, Duport Road, Eternal Love Winning Africa [ELWA], Rally Time, St. Joseph's, C.H. Rennie, and SOS) treating EVD survivors in Liberia.

Office of the Chief Scientist, Integrated Research Facility

Support Provided by the Clinical Monitoring Research Program

The mission of the Office of the Chief Scientist, Integrated Research Facility (OCSIRF) is to manage, coordinate, and facilitate the conduct of emerging infectious disease and biodefense research for the development of vaccines and medical counter-measures, and the improvement of clinical outcomes for patients. OCSIRF executes the biodefense research needed to understand clinical disease processes associated with the severity of microbial-induced diseases. Central to its core mission is the use of hospital tools, including endoscopy, cardiac telemetry monitors, computed tomography (CT), magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), and positron emission tomography (PET) imaging to evaluate pathogenic processes and disease trajectory in animal models exposed to microbes.

CMRP provides programmatic support and management oversight for research subcontracts to support OCSIRF initiatives. Support previously provided by Turner Consulting Group (TCG) under the auspices of its subcontract remains in a transition phase. The chief senior scientist, who previously supported a large portion of the TCG scope of work, has transitioned into a government position supporting NIAID. The TCG subcontract was extended for fiscal year 2016 as a no-cost extension in the event that support is requested. This extension will allow Leidos Biomedical Research to react quickly in response to the customer's needs.

Intramural Clinical Management Operations Branch

Support Provided by the Clinical Monitoring Research Program

The Intramural Clinical Management and Operations Branch (ICMOB) oversees the logistical management of clinical research and related clinical operations for the following National Institute of Allergy and Infectious Diseases (NIAID) intramural laboratories: the Laboratory of Immunoregulation (LIR), the Laboratory of Immunology (LI), the Laboratory of Host Defenses (LHD), the Laboratory of Clinical Infectious Diseases (LCID), the Laboratory of Infectious Diseases (LID), the Laboratory of Parasitic Diseases (LPD), and the Laboratory of Allergic Diseases (LAD). ICMOB manages one inpatient unit and two outpatient clinics at the NIH Clinical Center. The CMRP team is responsible for clinical protocol review and approval, assurance of scientific quality and human subject protection, the quality of care delivered to NIAID patients, and the quality of the professional performance of the health care providers.

The intramural portfolio constantly expands as new research initiatives and projects are identified to help further NIAID's research agenda. CMRP is actively involved with these mission-critical projects through the provision of clinical staff resources, such as clinicians, study coordinators, and administrative support personnel, as requested by NIAID.

CMRP provides six nurse practitioners who function as clinicians, managing acute and chronic diseases that are studied through NIAID protocols in both an inpatient and outpatient setting. Eleven protocol nurse coordinators provide direct protocol

management, ranging from recruitment and patient consent, to the collection and recording of research-driven data and the handling of regulatory reviews. Fourteen case managers provide nursing care to an assigned caseload of patients, utilizing the nursing process to assess, plan, intervene, and follow up on disease-related features as outlined in the clinical protocol. These staff members also coordinate and schedule patient visits to meet the required protocol procedures and data collection time points. Two clinical research nurses gather clinical information for prospective and current patients, in addition to helping with case management and monitoring activities pertaining to clinical protocols, including patient recruitment and retention, trial progress, and the need to extend or renew ongoing clinical trials. Four patient care coordinators organize the complex and comprehensive logistical needs of NIAID protocol patients by coordinating follow-up visits, diagnostic tests, and travel arrangements. One physician served as lead associate investigator on several protocols and provided outreach to a community clinic; however, that position was reclassified as a senior level nurse practitioner. Two physician assistants support clinic efforts by performing protocol-mandated initial and follow-up medical histories and physical examinations. A physician provides consultation in the area of infectious disease for a transplant program studying chronic granulomatous disease, in addition to supporting protocol and clinical operations. Collectively, the group ensures the conduct of high-quality clinical research through quality assurance activities and adherence to human subject protection guidelines; updates clinical staff on patient care, protocol process, and progress; and provides International Conference on Harmonization/Good Clinical Practices (ICH/GCP) and quality assurance (QA) education, while providing support to more than 60 protocols.

In addition to the credentialed clinical support staff, CMRP also provides patient education/ recruitment expertise. The patient educator/recruiter manages the placement and tracking of media advertising, medical chart reviews, phone screening, and community outreach, and serves as the major conduit through which referrals of prospective patient and normal volunteers will enter the clinical trials network. One research technician and one clinical research associate are responsible for collecting data, updating logs, and categorizing and preparing infectious and noninfectious tissue specimens for shipments derived from clinical research trials.

CMRP provided clinical nurse administrator support to ICMOB leadership and its core team until January 2016. The clinical nurse administrator served a quality role by reviewing documentation for those currently receiving inpatient care to ensure that all requirements are fulfilled for admissions and discharges. The clinical nurse administrator was also responsible for identifying and addressing any medical record delinquencies with appropriate NIAID-licensed independent providers, and communicating directly with ICMOB staff to ensure that any necessary follow-ups are addressed in a timely fashion. CMRP no longer provides this support to ICMOB's clinical teams.

While the U.S. Department of Health and Human Services (HHS) is responsible for maintaining a living document that provides federally approved HIV/AIDS medical practice guidelines, a number of CMRP staff members are providing technical writing and logistical support services to the HHS Panel on Antiretroviral Guidelines for Adults and Adolescents. This HHS panel has standing meetings that are conducted via teleconference, with one annual face-to-face meeting that, to minimize costs, is coordinated to coincide with the Annual Conference on Retroviruses and Opportunistic Infections. Several NIAID ICMOB staff members provide key leadership to the HHS panel. The work of the HHS panel affects NIAID's domestic and international HIV protocols, and the clinical staff members benefit from the panel's summary, which, in turn, benefits the HIV patients. CMRP directs the necessary resources to support all aspects of conference planning for the face-to-face meeting of the HHS panel. This support includes managing the necessary government approvals, planning and overseeing the budget, coordinating travel, facilitating hotel and meeting room arrangements, and providing on-the-ground support during the meeting. CMRP also provides oversight of a research subcontract for technical writing services for the HHS panel. These services include the provision of qualified personnel who have the necessary editorial and technical skills, as well as knowledge of scientific and medical terminology, to prepare minutes for the HHS panel teleconferences and the face-to-face meeting.

Research Subcontract Support

ICMOB is responsible for the quality of care delivered to NIAID patients and is accountable for the professional performance of the NIAID clinical staff. There continues to be a critical need to provide both NIAID and Leidos Biomed clinical staff that support NIAID with a mechanism to more efficiently earn continuing medical education (CME) credits to maintain required licensure and NIAID credentialing.

CMRP manages and administers a research subcontract with Professional Education Services Group (PESG) for accreditation services for seminars and other training type events attended by clinical staff. During this reporting period, the PESG agreement was extended to cover services through FY2016 and a modification to extend the agreement into the next fiscal year was issued prior to the end of the current fiscal year.

CMRP also manages and administers a Blanket Purchase Agreement (BPA) with Palladian Partners, Inc. to provide specialized science, medical, and public health communication support. Services provided by Palladian Partners include scientific writing and editorial support, quality assurance expertise, transcription, translations, and meeting support. Under the BPA, Palladian Partners provides the services of Dr. Frances McFarland Horne to support the NIAID/ICMOB/Office of AIDS Research Advisory Council (OARAC) Panel for Antiretroviral Guidelines for Adults and Adolescents. The Palladian Partners BPA period of performance was extended to continue through December 2016.

Laboratory of Allergic Diseases

The Laboratory of Allergic Diseases (LAD) conducts basic and clinical research on immunologic diseases, with an emphasis on disorders of immediate hypersensitivity, which include the spectrum of classic allergic diseases. The LAD scientific agenda is composed of basic and translational research aimed at elucidating events in mast cell–dependent, IgE-mediated allergic inflammatory reactions, including anaphylaxis, systemic mast cell disorders, and physical urticarias. Research efforts are focused on the role of mast cells, basophils, eosinophils, and T lymphocytes, and their cytokines in these disorders.

CMRP provides clinical support staff to assist in the conduct and facilitation of LAD's primary research objectives. The case managers are often sought to provide support to other research labs within the Division of Intramural Research (DIR), and this practice of leveraging clinical expertise from other laboratories maximizes the utilization of resources.

The registered nurse case management services include the management of protocols that study various aspects of allergic and inflammatory diseases, atopic dermatitis, urticarial syndromes, and systemic capillary leak syndrome (SCLS). One nurse case manager provides dedicated support to the natural history protocol studying diseases of allergic inflammation, focusing on subjects with moderate to severe atopic dermatitis, or with suspected genetic or congenital disorders associated with allergic inflammation. The nurse case managers conduct allergen skin prick testing, provide patient teaching for inpatient wet wrap therapy, educate all protocol patients about the eczema management plan, and serve as the point-of-contact for all protocol patients, fielding questions and concerns, scheduling post-treatment visits, and assisting with the coordination of travel and lodging arrangements. The nurse case managers maintain data collection and entry responsibilities for the quality of life questionnaires for all protocol patients.

In the past year, protocol continues to see genetics patients, specifically observing elevated tryptase families, implementing skin biopsies to look at differences in the mast cells in the electronmicroscopy, and looking at the constellation of symptoms that seem to be associated with elevated tryptase. In addition, vibratory testing has been implemented to study the reaction to vibrations that some patients are experiencing. For the atopic dermatitis patients, infrared imaging and cooling cuff testing has been initiated in collaboration with the National Institute of Biomedical Imaging and Bioengineering (NIBIB) to search for changes with inflammatory cytokines related to temperature.

Two CMRP nurse case managers in LAD provide direct nursing care to an assigned caseload of patients, utilizing a well-defined nursing process for assessing, planning, intervening, and following up on disease-related features as outlined in the clinical protocols. The nurse case managers also provide procedural support using various techniques for skin punch biopsies, allergen-antigen skin prick testing, and pulmonary function testing with impulse oscillometry. These clinical professionals operate with a unique combination of knowledge and abilities requiring not only nursing skills, but also an expert ability to coordinate a complex set of logistical and clinical variables unique to each protocol and patient.

CMRP staff support the following protocols:

- 02-I-0055: Evaluation, treatment, and follow-up of patients with Lyme disease.
- 96-I-0052: A comprehensive clinical, microbiological, and immunological assessment of patients with post-treatment Lyme disease syndrome and selected control populations.
- 09-I-N017: Research use of stored human specimens and/or data.
- 09-I-0126: Pathogenesis of physically induced urticarial syndromes.
- 09-I-0184: Studies in the pathogenesis of systemic capillary leak syndrome.
- 02-I-0277 Regulation of the proliferation and survival of normal and neoplastic human mast cells.
- 10-I-0148: Natural History of Atopic Dermatitis and other genetic/congenital diseases associated with allergic inflammation.
- 15-I-0159: Immunologic Effects of Supplemental Monosaccharide and Nucleoside Derivatives in Patients with Inherited Disorders of Glycosylation.

The nurse case manager who supports protocols 02-I-0055, 96-I-0052, 09-I-NO17, 09-I-0126, and 09-I-0184 is also tasked with a variety of responsibilities in support of the Outpatient 11 (OP11) clinic. These include maintenance, troubleshooting, and operations for patient testing, as well as oversight of the safe environment of care for allergy testing, which includes managing the secure storage of NIAID LAD and National Institute of Biomedical Imaging and Engineering instrumentation, and ensuring that all equipment is properly disinfected and inspected by NIH biomed services for safe use during direct patient care.

The nurse case manager performs the skin prick testing (SPT) procedures and facilitates the transport, maintenance, and storage of the allergen extracts as ordered by LAD principal investigators to ensure the LAD allergy consult physicians have immediate access to allergen testing supplies.

The nurse case manager is enrolled in Project Immune Readiness, a program supported by the U.S. Army Medical Department, as an online training student to ensure clinical expertise and safe conduct of practice; he also holds a National Institute for Occupational Safety and Health (NIOSH)–approved certification. Additionally, the nurse case manager maintains specialized training and skills to perform aeroallergen sampling data entry and microscopic identification of sampled aeroallergens obtained from Burkard and Rotorod air-sampling devices. Safe environment of care has been attained to 100 percent for direct patient care mission activities and procedures employing conscious sedation (i.e., esophagogastroduodenoscopies with biopsies). As a credentialed health care provider, this nurse case manager is involved in contributing to various knowledge-sharing efforts fostered by the LAD investigators, including manuscript development.

During FY2016, LAD saw patients with autosomal-recessive hypomorphic loss-of-function mutations in phosphoglucomutase 3 (PGM 3). These mutations have been shown to result in a novel congenital disorder of glycosylation (CDG), presenting with a hyper-IgE clinical phenotype. The goal is to understand how glycosylation defects result in atopic diatheses and immune dysregulation, providing novel insight into their immunopathogenesis. Developing successful therapies in these patients may further provide novel targets or approaches to the treatment of allergic diseases in the general population.

For the 15-I-0159 protocol, the case manager developed several aids to assist patients: (1) an instruction sheet that describes how to properly take the sugar uridine supplement and informs about potential side effects, (2) a letter for patients to carry when traveling, showing principal investigator (PI) authorization to carry the white powdered sugar supplement, and (3) a notebook for the patients to keep their protocol event logs.

In addition to supporting LAD investigators with other activities such as medication inventory documentation and cold chain storage policy guidelines, CMRP staff also collaborate with PIs in other labs to support LCID and LPD clinical research studies.

Laboratory of Clinical Infectious Diseases

NIAID's LCID conducts clinical and basic studies of important human infectious and immunologic diseases, focusing on mycobacterial, bacterial, viral, and fungal infections, as well as the acquired and congenital immune disorders associated with infection, susceptibility, and resistance. CMRP staff members supporting LCID are involved in the study and treatment of a wide spectrum of diseases, including primary immunodeficiencies, hyper IgE syndrome, herpes simplex virus, tick-borne infections, and autoimmune lymphoproliferative syndrome, as well as the identification of novel viruses. This report discusses support to the autoimmune lymphoproliferative syndrome (ALPS) Unit, which is studying and identifying rare immune disorders to better understand the genetic physiology of the known and newly identified rare immune disorders, as well as support to the Lyme Disease Research Program, which is working to develop better means of diagnosing, treating, and preventing Lyme disease.

The ALPS Unit focuses on gaining a better understanding of the clinical and genetic characteristics of people with ALPS and related disorders. By identifying the genes responsible for symptoms, NIAID researchers not only help affected families, but also increase the understanding of how the immune system works. Ion Torrent sequencing from the NIH Department of Laboratory Medicine is one of the tools the investigators are using to address these research questions. Ultimately, they hope to develop safe and effective treatments targeting the genetic defects in children with ALPS and related disorders.

There has been an increase in the number of referrals to the team. The CMRP clinical research nurse continues to obtain records, pathology material, and radiology CDs prior to a patient's first visit and for interim visits. The clinical research nurse also obtains informed consent before any screening tests are done, enrolls patient information on the Clinical Research Information Management System of NIAID (CRIMSON), and ensures specimens are sent to the appropriate locations for testing.

The clinical research nurse provides support to the ALPS team primarily at the direction of the ALPS study coordinator. This support includes referring new patients for consideration, loading active and new patient records into CRIMSON, processing blood and saliva samples, processing pathology slides, filing research reports in secure electronic files, processing outside radiology films, sending patient visit reports to local physicians, and converting old paper files to secure electronic files.

With the implementation of protocol 15-I-0135 (an open-label, non-randomized, within-patient dose-finding study followed by a randomized, double-blind, placebo-controlled study to assess the safety and efficacy of CDZ173 in patients with APDS/PASLI (Activated Phosphoinositide 3-kinase D), the clinical research nurse assists the designated study coordinator by scanning and printing documents. When the patients are seen, their clinic reports are sent to the local doctors.

With the availability of NIH Secure Email, the clinical research nurse is inviting local physicians to utilize that system for the exchange of patient information instead of the traditional email or fax systems. To date, nearly half of the physicians approached have accepted the system.

The NIAID Lyme disease research portfolio includes a broad range of activities designed to increase understanding of this disease through basic and clinical research studies conducted by extramural and intramural investigators.

The CMRP nurse practitioner assists in reviewing potential future candidates for the Lyme Disease Research Program. A portion of requests for study participation is submitted to the nurse practitioner, who reviews the accompanying data for eligibility. The nurse practitioner reviews the data and contacts the potential participant to conduct a telephone interview to obtain more-detailed clinical information. The nurse practitioner presents clinical cases at the weekly program meetings. Patients determined to be eligible are seen by the nurse practitioner in clinic for medical history, physical exam, and skin biopsies (as necessary). The nurse practitioner also helps to ensure that all protocol timelines for return visits are met. Additional support activities include seeing patients for initial and follow-up visits, cosigning clinical notes from other team members involved in the care of patients on specific clinical trials, participating in the clinical meetings with patients to summarize their specific clinical findings and treatment plans, consulting with specialists in different departments, following patient lab results, and communicating any pertinent abnormal results to staff physicians.

Laboratory of Host Defenses

The Laboratory of Host Defenses (LHD) studies the immune functions essential for the host's defense against infection. LHD also studies the genetics and pathophysiology of inherited primary immune deficiencies. These abnormalities may be associated with recurrent infections and/or dysfunctions of immune homeostasis, which the lab investigates through clinical protocols.

LHD clinical investigations aim to develop new diagnostic and therapeutic approaches to the management or correction of immune dysfunction in patients. CMRP provides a variety of clinical staff to support LHD, including protocol nurse coordinators, nurse case managers, a clinical research nurse, and a physician. The major areas of research for LHD include the study of gene therapy, inflammatory bowel disease (IBD), common variable immune deficiency, chronic granulomatous disease (CGD), allogeneic transplantation using hematopoietic stem cell grafts, and acute and chronic graft-versus-host diseases.

The protocol nurse coordinators support LHD by reviewing activities related to screening and enrolling new subjects. In addition, they oversee the lab's regulatory requirements by completing continuing reviews and protocol amendments. In addition to regulatory support, the protocol nurse coordinators also provide research support by assisting with specimen collections and scheduling subjects for various clinical and research–related procedures.

During FY2016, a protocol nurse coordinator spearheaded an effort to revitalize enrollment on an existing protocol that included a new cohort of subjects. This effort has increased enrollment, decreased visit follow-up times, and included the implementation of a tracking mechanism to ensure that patients are not lost to follow-up. The protocol nurse coordinator created a new screening tool to streamline the screening process and also helped to develop a new LHD protocol for a Phase I/II open label pilot study to assess the safety and tolerability of Vorinostat for the treatment of moderate to severe Crohn's disease.

The clinical research nurse supports LHD by coordinating the review and screening of subjects across two protocols. She works closely with the study team to schedule and organize visits for new and returning subjects. In addition, the clinical research nurse also schedules and transports research specimens to ensure that protocol integrity is maintained.

The LHD nurse case managers work closely with team members to successfully bring patients to the NIH Clinical Center. The nurse case managers serve as the point of contact for the study patients, often fielding questions and concerns, as well as scheduling and coordinating study visits and post-treatment follow-ups.

The CMRP physician supports protocols and clinical operations initiated by LHD. The patient population is complex, and includes children and adults with congenital immunodeficiencies, such as CGD of childhood, X-linked severe combined immunodeficiency (XL-SCID), and X-linked agammaglobulinemia. Since these conditions predispose these patients to complex, recurrent, and chronic infections, an infectious diseases physician is a major asset to their care. The physician is active as the vice chair of the Institutional Review Board (IRB) for NIAID, assuming responsibility for protocols from a selection of the NIAID laboratories.

LHD also supports a transplant program for CGD and XL-SCID, and is currently involved in gene therapy for XL-SCID, CGD, allogeneic transplantation using hematopoietic stem cell grafts, and acute and chronic graft-versus-host diseases. The physician supports the primary care, infectious disease consultation, and transplant-related activities related to new immunodeficiencies such as DOCK-9, PASLI, and WHIM syndrome. The physician continues to participate in in-house and long-distance consultation (for patients not yet enrolled, or those enrolled but receiving care at outside facilities) for patients with congenital immunodeficiencies beyond the scope of transplantation infectious diseases. Other new responsibilities are related to the transition to the LHD of patients previously seen in the National Human Genome Research Institute (NHGRI) with Wiskott-Aldrich syndrome and adenosine deaminase severe combined immunodeficiency (ADA-SCID), some of whom received transplants.

The physician provides consultation support in the pre-transplantation evaluation, and the care related to complications that may occur during and/or after the transplant. In addition, the physician participates in the infectious diseases practice at the NIH Clinical Center by being involved in the care provided by the infectious diseases consult service, and in the education of students, residents, and infectious disease fellows. To facilitate the consistent practice of transplant infectious diseases medicine, the physician has leveraged the opportunity to observe and provide consultation in the NCI Experimental Transplantation and Immunology Branch (ETIB) program. These efforts are directly related to improving the consistency of care at the NIH Clinical Center associated with the management of the infectious complications of transplantation. Additional responsibilities and opportunities have arisen that are related to transplantation and post-transplant care for patients with other complex immunodeficiencies. Experience with this broader population provides additional information and increases interaction with the NIH transplant community, which is vital in understanding the alternative approaches to infectious and noninfectious diseases associated with transplantation. In addition, the physician's presence has added sufficient supervisory capacity to improve LHD's continuity of care in providing ongoing treatment of the large cohort of immunodeficiency and post-transplant patients so that LHD care is reviewed on a weekly basis with LHD staff. The CMRP physician serves as the attending of record when principal investigators are absent.

Laboratory of Infectious Diseases

The Laboratory of Infectious Diseases (LID) has a long history of vaccine development and identification of new agents of viral diseases. LID undertakes high-risk, high-reward programs that require extraordinary time and resource commitments, such as programs to develop vaccines for viral hepatitis, severe childhood respiratory diseases, and viral gastroenteritis. Clinical studies complement LID's major areas of research, including testing candidate vaccines in clinical trials, a human challenge study with influenza to study pathogenesis and immune correlates for protection against the virus, and studies of severe virus infections in persons without known immune deficiency.

LID sees patients for initial and follow-up visits. The CMRP Nurse Practitioner (NP) provides patient care according to best clinical practices and protocol guidelines as outlined by LID. She works with specialists from other departments and with both the clinical and non-clinical support staff of the Clinical Center to meet LID's needs and goals.

During FY2016, the NP conducted case reviews of potential future candidates, handled more than 100 patient visits for history and physical exams and/or vaccine administration, and worked to ensure that all protocol timelines for return visits were met.

The CMRP NP continued to see patients on natural history studies, performing histories and physical exams, reviewing labs and imaging results, and documenting visit details. During FY2016, the NP also began to see patients on a new respiratory syncytial virus (RSV) challenge study; she screened and evaluated participants for eligibility.

The CMRP NP also serves as a clinician on the inpatient Special Clinical Studies Unit (SCSU) at the Clinical Center, works with the LID clinical staff, and participates in the clinical meetings with patients to summarize their specific clinical findings and treatment plans.

Laboratory of Immunoregulation

The Laboratory of Immunoregulation (LIR) investigates the cellular and molecular mechanisms regulating the human immune response in health and disease. A major component of these efforts is the study of the immunopathogenic mechanisms of HIV infection and disease progression. Developing a thorough understanding of how to prevent and treat HIV infection requires an understanding of how HIV destroys the immune system. Several important aspects of this process are under investigation and CMRP plays a significant role in providing the clinical support resources needed to drive progress and achieve success.

CMRP supports all aspects of research in LIR/OP8 by providing two licensed independent practitioners, three study coordinators, one case

manager, one clinical research associate, one research technician, and one recruiter. Three of the 14 full-time LIR/OP8 study coordinators are Leidos Biomed employees. During FY2016, LIR supported over 60 active protocols. In addition to mission-sensitive, HIV-related research, LIR placed great emphasis on domestic and international Ebola research efforts. CMRP staff also worked with LIR physicians to develop new studies, including a natural history protocol for the Zika virus.

Overall, the CMRP staff worked to meet enrollment goals across several high-priority studies and served as resources for the international Ebola vaccine and treatment trials while continuing to maintain existing long-term follow-up studies in the outpatient setting.

In January 2016, the NIAID IRB approved an investigational new drug (IND) Phase I study (# 16-I-0044) of CC-11050 in HIV-1-infected adults with suppressed plasma viremia on antiretroviral therapy. To help meet the demands of the enrollment deadline, the protocol nurse coordinator collaborated with staff from local hospitals to identify potential subjects. These efforts increased enrollment for subjects who are local to the NIH area, decreasing the amount of the NIAID travel budget spent on subjects who live further than 50 miles away from the Clinical Center. Additionally, the CMRP recruiter worked closely with the Patient Recruitment and Public Liaison office and the Office of Communications and Government Relations to disseminate information about upcoming studies through the NIH listserv and various social media platforms. The patient recruiter also uses Research Math, a subscriber-based service that matches interested study participants to appropriate protocols, for many healthy volunteer studies. The recruiter typically pre-screens approximately 60 potential research participants per month. Of note, almost 100% of potential LIR/OP8 research participants are filtered through this recruiter.

The nurse case manager took an active role in developing and implementing a clinic-wide initiative to improve the communication between nursing staff when transitioning care from one staff member to another case manager. This process resulted in the development of a standardized tool for hand-off communication, thereby preventing communication errors that could negatively impact patient care.

The CMRP research technician was asked to serve as the head WatSan, responsible for coordinating WatSan volunteers at the Clinical Center when a patient suspected with Ebola virus disease arrives at NIH. The WatSans ensure staff properly don and doff personal protective equipment.

CMRP staff also supported publication activity during FY2016. The protocol nurse coordinator supported a journal article on the use of antiretroviral therapy in early asymptomatic HIV infections in the *N Engl JMed*, and the research technician co-authored a manuscript regarding the effects of tenofovir on bone and renal health in HIV-infected young adults.

Laboratory of Parasitic Diseases

The Laboratory of Parasitic Diseases (LPD) conducts basic and applied research on the prevention, control, and treatment of a variety of parasitic and bacterial diseases of global health importance; this includes both laboratory and patient-centered research conducted at the NIH Clinical Center. Research efforts are largely focused on the identification of immunological and molecular targets for disease intervention. LPD also conducts research related to eosinophils' function and activation and the role of eosinophils in disease pathogenesis, with the ultimate goal of developing novel diagnostic tools and treatments for hypereosinophilic syndromes and other conditions with marked eosinophilia, including helminth infections. CMRP provides physician assistant support, nurse case management support, and study coordination services to LPD.

Collectively, the support staff members coordinate patient schedules, schedule protocol-mandated tests and procedures, obtain data, perform initial assessments, and provide nursing care. CMRP staff participate in the development of protocols, procedure manuals, and case report forms (CRFs). Additionally, these staff members oversee protocol operations to ensure study compliance, troubleshoot possible protocol violations, and interface with the IRB to ensure the proper and timely filing of serious adverse events (SAEs), amendments, annual reports, and other regulatory documents, including biweekly safety reports. CMRP staff members play an active role in providing updates to clinical teams on patient care and on protocol progress.

The overall volume of patient visits continues to be high. While none of the LPD studies have a large number of subjects enrolled, each protocol requires frequent follow-up visits. As a result, the case managers and protocol nurse coordinators are scheduling in excess of 80 patient visits per month on average.

The protocol nurse coordinator, who serves as an associate investigator on the active protocols within LPD, assisted investigators in collecting data for patients with eosinophilia. These efforts led to two manuscript publications, and a poster presentation at an international conference.

Screening and enrollment continued for the following ongoing IND studies: 14-I-0063, An Open-Label, Proof-of-Concept Study to Evaluate the Safety and Efficacy of Dexpramipexole (KNS-760704) in Subjects with Hypereosinophilic Syndrome; and 14-I-0081, A Phase 2a Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Subcutaneous Benralizumab (MEDI-563)

in Reducing Eosinophilia in Subjects with Hypereosinophilic Syndrome (HES). Both of these protocols are expected to be fully enrolled by the end of FY2016. The tenth and last subject on IND protocol 14-I-0115, A Double-Blind, Randomized, Placebo-Controlled Study to Investigate the Efficacy and Safety of Mepolizumab in the Treatment of Eosinophilic Granulomatosis with Polyangiitis (EGPA) in Subjects Receiving Standard-of-Care Therapy, will complete the study before the end of FY2016.

During FY2016, LPD initiated one new IND protocol titled "Mepolizumab Long-Term Access Programme for Subjects who Participated in Study MEA1155921 (Placebo-controlled Study of Mepolizumab in the Treatment of Eosinophilic Granulomatosis with Polyangiitis in Subjects Receiving Standard-of-care Therapy)." This study began enrolling subjects in December 2015. The protocol nurse coordinators have been collaborating with the gastroenterologist at the National Institute of Diabetes and Digestive and Kidney Diseases to develop a new microbiome project in eosinophilic subjects. This will allow both groups to study the microbiome effect in eosinophilic patients with GI involvement through the use of food diaries, stool analysis, and various invasive procedures.

Pharmacy Support

The CMRP clinical pharmacist provides pharmacologic support to NIAID's clinical operations. This support includes patient education, inpatient and outpatient clinic services, research-related support, and pharmacologic guidance and expertise. The clinical pharmacist works with patients to ensure their understanding of antiretroviral therapies, opportunistic infection (OI) treatment, HIV pathophysiology, and treatment goals. Hospital inpatient and outpatient clinic services include discharge counseling, continuity-of-care between inpatient and outpatient settings, support to rotating medical residents involving inpatient HIV patients, support to NIAID Fellows while on HIV clinic assignments, and support to NIAID HIV attending and clinic physicians involved with antiretroviral regimens and OI treatment/prophylaxis.

Specifically, the clinical pharmacist assists with entering orders for take-home medications and verifying order accuracy; serves as a liaison to the NIH Clinical Center pharmacy staff; follows up on labs and medication orders for HIV patients, providing recommendations as necessary; provides pharmacologic expertise involving dosing, drug interactions, and side effects of antiretroviral, anti-hepatitis C, and concomitant medications; and follows up with addressing adherence barriers to therapies. Medication adherence support includes coordination with the outpatient pharmacy, pillbox teaching, and communication with caregivers and assisted living staff. All services are provided to ongoing natural history protocols for HIV, hepatitis C, and pharmacokinetic studies.

The clinical pharmacist acts as an observer on the HHS panel, provides drug interaction tables for the HHS HIV guidelines, and conducts literature reviews upon request. The clinical pharmacist also provides logistical support for annual meetings. Additionally, this staff member serves as a mentor for pharmaco-kinetics research fellows, provides lectures to the NIAID fellows on HHS guidelines and antiretroviral therapy, and provides nursing and physician assistant education on antiretroviral therapy.

The clinical pharmacist aided the preparation of the 2016 update to the HHS guidelines on the use of antiretroviral agents in HIV-1 infected adults and adolescents. This included updating the drug-drug interaction tables as well as preparing a summary of evidence on when to start antiretroviral therapy in patients concomitantly infected with tuberculosis.

The clinical pharmacist initiated bi-weekly educational sessions with the HIV clinic physician assistants, prepared presentations on HIV treatment topics to help orient new clinical support staff without HIV-specific experience about potential clinical challenges in the research clinic, and routinely provided HIV education to new nurse case managers and study coordinators.

To support the NIH OP-8 HIV Clinic, the clinical pharmacist continued to monitor and update the CRIMSON medication database, which is essential to capturing data on patients' medication use.

The clinical pharmacist began work with Dr. Henry Masur to present potential clinical queries on the D.C. cohort of a joint research project between the George Washington University Milken Institute School of Public Health and NIAID. Over 7,000 HIV+ patients have enrolled into the D.C. cohort and the clinical pharmacist is working with the George Washington University team to identify retrospective data reviews that may lead to publication and quality control efforts at the HIV clinics.

The clinical pharmacist prepared Pharmacy and Therapeutics Committee formulary requests for the following antiretroviral drug combinations: Odefsey, Genvoya, and Descovy, and aided the clinic staff in transitioning patients to these new medications after the NIH pharmacy had obtained less expensive Department of Defense contract pricing when feasible. The clinical pharmacist also brought beclomethasone, an inhaled corticosteroid, to the Pharmacy and Therapeutics Committee for addition to the formulary. The addition of this drug to the Clinical Center's pharmacy will allow patients who require an inhaled corticosteroid to use one that has been shown to not interact with anti-HIV medications, unlike those currently available at the Clinical Center.

Collaborative Clinical Research Branch

Support Provided by the Clinical Monitoring Research Program

The Collaborative Clinical Research Branch (CCRB) facilitates high-quality clinical research in infectious diseases through active participation in selected domestic and international collaborations. CCRB plays an integral role in the National Institute of Allergy and Infectious Diseases (NIAID) mission, advancement of the U.S. Department of Health and Human Services (HHS) Global Health Agenda, and execution of special projects related to HIV/AIDS, biodefense, and infectious and immunologic diseases. A CMRP physician and clinical project manager (CPM) support CCRB activities by providing technical expertise for the implementation and management of research strategies, developing and teaching sound clinical concepts related to infectious disease research, and by presenting and publishing valuable information to the research community. These specialized persons provide project and program management for Division of Cancer Research (DCR) projects, coaching, and mentoring to the next generation of clinical researchers, as well as clinical and scientific consulting services for NIAID's portfolio of mission-critical clinical research initiatives.

Technical and Scientific Support

The CPM provides operational leadership, coordination, and overall management of multiple special projects. The CPM's technical expertise contributes to the development, implementation, and management of project strategies by developing goals and objectives, formulating and monitoring operational metrics, and identifying risks and mitigation strategies. Working collaboratively with the CCRB and CMRP program and project leadership, the CPM helps to set operational standards, identify and address project barriers, establish project communication strategies, direct study teams and drive accountability, and identify process improvements. During this past year, the CPM supported the INA-RESPOND Data and Safety Monitoring Board (DSMB) executive secretary in updating the DSMB charter and with training and mentoring support for the DSMB.

The CPM is actively involved in the South East Asia Infectious Disease Clinical Research Network (SEAICRN), Indonesia Research Partnership on Infectious Diseases (INA-RESPOND), and Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) studies. In conjunction with CMRP senior leadership, the CPM recruited and hired a new project manager to assist the technical project manager with subcontracting oversight responsibilities for NIAID's INA-RESPOND clinical research network. Additionally, the CPM served as a member of the transition team for the introduction and orientation of the newly awarded subcontractors to provide in-country support to the INA-RESPOND clinical research network. The CPM contributed to the agendas for the SEAICRN and INA-RESPOND annual meetings and participated in the overall planning and execution of those meetings. The CPM also prepared and attended the transition kick-off meeting for the new subcontractors. In addition, the CPM, the SEAICRN medical officer, and the in-country team focused efforts on the SEAICRN initiative by providing technical support and building relationships through various mechanisms. The CPM continued to work closely with the clinical research associates and NIAID clinical research oversight manager to review and provide input to the monitoring reports. The CPM provided guidance and oversight for the development and finalization of the INA-RESPOND network-specific standard operating procedures (SOPs), guidance documents, and supporting documents for the INA-RESPOND Secretariat. The CPM and team visited two sites in Indonesia to assess for project progress and Good Clinical Practices (GCP) competency. The CPM attended and actively participated in all of the Executive Committee meetings and several Governing Board meetings for both the SEAICRN and INA-RESPOND networks, helping to define the future vision for INA-RESPOND and close out the SEAICRN Network. The CPM has taken on the oversight of the data management and IT transition from the previous contractor to the International Biomedical Research Support Program (IBRSP), Office of Cyber Infrastructure and Computational Biology (OCICB), National Institutes of Health: National Institute of Allergy and Infectious Diseases (NIH/NIAID), and the INA-RESPOND staff. The CPM worked with the deputy branch chief, the previous contractor, and the OCICB team to implement a plan through June 2016 and preserve the integrity and safety of the data.

The CPM worked with the site specialists and monitoring staff to initiate three of the five sites participating in the Tuberculosis Research of INA-RESPOND on Drug Resistance (TRIPOD) study, a multi-center cohort study of drug resistance in tuberculosis (TB). The CPM has also been working with the in-country protocol team to develop the newest INA-RESPOND protocol "Proactive," which is a prospective observational cohort study of HIV infection and risk-related coinfections/ comorbidities in Indonesia.

The CPM has been flexible within the branch to support initiatives for bridging communications across NIH and NIAID divisions, such as attending and participating in the monthly International Coordinating Committee (ICC) and the monthly Inter-Institute Bioethics Interest Group. For the

PREVAIL project, the CPM supported the in-country teams as needed for the Ebola natural history study. This entailed taking a lead role to facilitate activities to assure the study staff had the necessary supplies, facilities, and support.

The CPM, in conjunction with other CMRP project managers, is helping to develop a webinar for the Association of Clinical Research Professionals (ACRP) for a meeting and expo session later in 2016. The topic will discuss supporting Ebola clinical research activities in West Africa as an informative webinar for the entire ACRP membership base.

Clinical Consulting Support

The CMRP physician serves as a clinical attending on the inpatient infectious diseases consult service at the NIH Clinical Center. In this capacity, the physician engages in patient care and teaches medical students, residents, and Fellows. The physician coaches the incoming NIH clinical Fellows on clinical research design and mentors the Fellows to enhance their clinical research capacity. The physician also attends a biweekly journal club and instructs Fellows and research staff on design, analysis, and interpretation of clinical research studies.

The physician serves as a mentor and co-investigator with NIAID and NIH clinical Fellows on various research efforts, including: (1) evaluation of patients' experience of fever with the NIH Clinical Center nursing department; (2) evaluation of fungal clearance as a surrogate endpoint in crypotococcal meningitis, with NIAID Fellows and attending staff; (3) evaluation of natural history and outcomes of bacteremic patients in the Clinical Center population, with NIAID Fellows and attendings; (4) performance of discordant minimum inhibitory concentration analysis as a measure of drug effect, with the NIAID biostatistics department; (5) systematic review of brucella arteritis, with NIAID Fellows; and (6) evaluation of consent forms for clinical trials of antibiotics, with the NIH nursing department.

The physician continues to teach the Uniformed Services University (USU) introduction to clinical research course annually, deliver several lectures on clinical research designs, and provide lectures specific to clinical research methods for USU medical students.

The physician is a member of several national and international committees, bringing knowledge of current clinical research topics to the CCRB. The physician participates in these committees in varying capacities, such as: (1) member, Foundation for NIH (FNIH) working group on endpoints for skin infections, community-acquired pneumonia, and hospital-acquired pneumonia; (2) chair, International Society for Pharmacoeconomics and Outcomes Research (ISPOR) Clinician Reported Outcome task force (received award from U.S. Public Health Service); (3) adviser, World Health Organization (WHO) Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR); (4) member, Data and Safety Monitoring Board (DSMB) for two Division of Microbiology and Infectious Diseases (DMID) protocols on randomized double-blind trials evaluating older generic drugs (clindamycin and trimethoprim-sulfamethoxazole) for skin infections and skin abscess; and (5) full member, Department of Veterans Affairs, and Cooperative Studies Scientific Evaluation Committee (CSSEC).

During FY2016, the CMRP physician also assisted with the development of an emerging infectious disease protocol in Mali and with the analysis and development of a manuscript of data on causes of fever in Indonesia.

Infectious Diseases Clinical Research Program, Department of Defense

Since 2005, CMRP has worked with NIAID to establish and maintain a collaborative effort between CMRP, NIAID, and the Department of Defense (DoD) within the Infectious Diseases Clinical Research Program (IDCRP). With a CMRP physician serving as the team leader for the project, the overarching goal of this collaboration has been to facilitate high-priority, translational clinical research to address infectious disease problems of military relevance. Additional objectives include building research capacity, developing infrastructure, facilitating efficient clinical research, and leveraging scientific expertise within and outside of NIH.

The CMRP physician continues to facilitate the development of research capacity by aiding IDCRP staff in developing and implementing protocols, assisting with prioritizing research protocols within the network, and developing research areas of prime importance to the network. Regulatory Compliance and Human Subjects Protection Program (RCHSPP) staff has helped IDCRP develop an informed, independent staff for regulatory and monitoring functions. Under this mentorship, IDCRP staff members now monitor several of their own protocols, and have developed quality assurance and quality control standards for IDCRP.

The CMRP physician is the chair of the IDCRP Scientific Review Board (SRB), reviewing all new and amended research protocols for scientific design and validity, and chairing the review groups for each protocol. The physician also serves as an ex officio member of the IDCRP Steering Committee, which evaluates new research concepts before proceeding to protocol development, and is a member of the Senior Advisory Group, which reviews program operational progress and addresses challenges.

CMRP staff members have also helped develop research capacity by acting as points of contact for clinical research questions and standards, such as

NIAID-specific protocol templates and SOPs. Additionally, the CMRP physician has lectured groups of principal investigators and mentored individual principal investigators (PIs) to enhance their scientific understanding, and has worked closely with IDCRP staff to reorganize the data collection and analysis branch.

During FY2016, the CMRP physician aided DoD staff in maintaining an infectious disease–specific institutional review board and continued efforts with IDCRP staff on developing a study to evaluate various interventions in the treatment of diarrhea in military personnel in Europe, Africa, and the Middle East. RCHSPP staff provided protocol pre-review for regulatory compliance and protocol monitoring, when appropriate, per GCP regulations.

The CMRP physician continued assisting NIAID with completing the annual interagency agreement (IAA) to provide annual funding for the program. The IAA between NIH and IDCRP is renewed yearly and requires modifications that reflect changes within the organization. The CMRP physician is responsible for ascertaining any changes, working with NIAID's DCR to obtain appropriate approvals as needed, and ensuring that funds have been advanced as required by contract.

The CMRP physician continues to guide this project, often assisting and advising on the structure of new projects and guiding military physicians on the conduct of high-quality science, along with providing lectures to scientific groups and young investigators. The continuation of weekly and monthly meetings keeps CMRP staff informed of potential changes and ongoing needs for assistance and support. The CMRP physician routinely reports and discusses updates and changes with the NIAID clinical director.

Biostatistics Research Branch

Support Provided by the Clinical Monitoring Research Program

The mission of the Biostatistics Research Branch (BRB) is to develop collaborative relationships with intramural and extramural researchers and to conduct independent research in statistical methodology. CMRP staffs four biostatisticians to support this effort.

CMRP biostatisticians provide statistical and mathematical support, as well as data management, programming, and statistical data analysis to many intramural clinical research protocols. The biostatisticians also analyze novel, high-dimensional immune assay data collected through the Phase I vaccine studies conducted at NIAID's Vaccine Research Center (VRC), including vaccine efficacy and studies of malaria and HIV. In addition, the biostatisticians help develop and test novel statistical methods for researchers, assist in safety evaluations, and prepare Data and Safety Monitoring Board reports.

During FY2016, the biostatisticians were involved in a variety of projects, from developing analysis plans to performing complex statistical analyses, writing reports, and co-authoring manuscripts. Some of the projects supported by BRB included malaria, IRIS, autoimmune lympho-proliferative syndrome, and Food and Drug Administration (FDA) studies; NexGen early bactericidal activity and Predict TB trial; and influenza-like illness, autoimmune diseases, H1N1 flu, cryptococcal meningitis, and various vaccine studies. The biostatisticians also conducted various statistical tests, including descriptive statistics to regression models, graphs, and reports for several VRC studies (e.g., VRC 315, 208, and 308).

One of the biostatisticians continued to be directly involved in performing research experiments, data collection and processing, and assisting with the experimental imaging of simian immunodeficiency virus (SIV)/simian/human chimeric immunodeficiency virus (SHIV) in rhesus macaques.

Additional activities included: (1) analyzing data from noninvasive in vivo single-photon emission computed tomography and positron emission tomography imaging of SIV/SHIV-infected nonhuman primates; (2) testing new antibodies for noninvasive in vivo imaging; (3) studying the in vivo recovery of the immune systems of healthy monkeys that underwent total body irradiation or other conditioning regimens; and (4) designing in vivo studies targeting a cure for HIV.

Office of Planning and Operations Support

Support Provided by the Clinical Monitoring Research Program

The mission of the Office of Planning and Operations Support (OPOS) is to provide services and innovative solutions that optimize the facilitation of National Institute of Allergy and Infectious Diseases (NIAID) clinical research and special projects. CMRP provides executive leadership and management oversight for a variety of programmatic and administrative resources in support of this mission. These services include strategic and operational planning, learning and professional development, technical solution support, and research subcontract management and oversight.

Multiple CMRP staff members support the portfolio of services offered by OPOS. A program manager administers and applies project management principles and concepts, focusing on strategy implementation and performance management. A clinical training manager and clinical training specialist serve as the primary resources facilitating

and conducting learning and professional development activities supported by OPOS. A special projects administrator acts as the point of contact in the Technical Solutions Group, providing technical support to the branches and offices of NIAID Division of Cancer Research (DCR). These resources provide a breadth and depth of experience and knowledge that is leveraged throughout the division.

CMRP provides management oversight to several research subcontracts in support of OPOS program operations and major initiatives. These research subcontracts supply technical resources that provide facilitation experts for OPOS' ongoing organization development and strategic implementation initiatives, as well as leadership coaching services to support DCR's division-wide leadership enhancement initiative.

CMRP manages an organizational development consultant, Ellen Cull, who provides support to the Strategic Management in Clinical Research Networks in collaboration with another subcontractor, Jerry Lassa (referenced in the Performance Measures and Clinical Consulting and Support sections of the CMRP Annual Report). Both Ellen Cull and Jerry Lassa also support other special projects initiatives, which are outlined in the Clinical Consulting and Support section of this report.

Project Management Support

OPOS provides strategy and operational management for the DCR branches, offices, and special projects that ultimately further NIAID's clinical research agenda by setting and monitoring strategic goals and initiatives aligned with NIH priorities. CMRP provides a project manager to support the Organizational Effectiveness Group (OEG) and Special Projects Group (SPG) for OPOS. The project manager, in collaboration with the OPOS groups, provides strategy, operational, and project management support to NIAID's DCR.

In FY2016, the project manager worked to operationalize OPOS' new strategic plan, developed five new strategy management tools and guidelines, created four strategy management templates, and completed 21 progress reports for four branches/offices and two special projects.

The project manager continues to establish, implement, and maintain a flexible reporting system for monitoring the progress of operational plans, which requires facilitating the ongoing review and maintenance of DCR operational plans and special project operational plans, as well as progress reports for each branch/office/special project's leadership on an agreed-upon basis.

During this reporting period, quarterly progress reports were prepared for OPOS, the Office of Clinical Research Policy and Regulatory Operations (OCRPRO), the Program Planning and Analysis Branch (PPAB), the Indonesia Research Partnership on Infectious Diseases (INA-RESPOND), and the University Clinical Research Center (UCRC) in Mali. In addition, an annual report was prepared for the Biostatistics Research Branch (BRB).

To operationalize OPOS' new strategic plan, the project manager worked with the members of the strategic planning group to formulate a plan to communicate OPOS' strategy management efforts to OPOS staff, disseminate customized versions of the operational plan for functional groups and individuals, and collect updates to create progress reports for OPOS' new strategy. Additionally, the project manager worked with functional groups to modify objectives, tasks, and metrics as needed, and to identify targets for 2016. To further execute the strategy, the PMII developed an OPOS knowledge-sharing tracker to document publications, posters, and presentations; established knowledge-sharing guidelines; and created a new progress report template.

To evolve and align DCR's strategy management reporting dashboards, the project manager worked with other colleagues to pilot the development of a web-based DCR dashboard. This dashboard included measures from three DCR branches/offices, with the ability to sort by measure type, owner, and progress status. Once complete, the dashboard will be shared with leadership to determine the next steps.

The project manager continued to play a vital role in identifying and prioritizing strategy management tools for each of DCR's strategy management stages. The project manager gathered examples, developed sample templates, and created guidance tools—all of which will be part of a larger library of tools to support DCR's strategy management process. The project manager also continued to assist in formatting and finalizing DCR's strategy management process guidelines and two strategy management guides.

The project manager supports both UCRC and INA-RESPOND in their strategy management efforts. For the UCRC, the project manager participated in ongoing operational meetings to collect task updates and prepare progress reports on a quarterly basis. The PMII also co-facilitated a meeting with an OPOS colleague and the Mali project team lead to review DCR's best practices in strategy management, with a focus on how to communicate UCRC's strategy to all levels of the organization.

For INA-RESPOND, the project manager assisted with developing a new strategic goal, and identified potential objectives, tasks, and metrics. This proposed goal was shared with and adopted by the network, resulting in updates to all relevant strategy management documents to reflect the change. The project manager also created annual accomplishment reports for both UCRC and INA-RESPOND that highlighted the network's accomplishments as they relate to its strategic goals.

The project manager participated in the Strategic Management Research Project Team (SMRPT) that aims to identify how network strategy is being

managed, as well as ways to enhance strategy development, implementation, and adaptation for continuous improvement. The project manager was identified as a consultant on the INA-RESPOND project and provided the SMRPT strategy management documents and related materials. In addition, the project manager participated in two interview sessions where the team asked clarifying questions about developing and planning the network's strategy, aligning the network and plan operations, and monitoring/adapting the strategy.

In addition to supporting OPOS' strategy management activities, the project manager is responsible for assisting with DCR's workforce alignment efforts. Utilizing a performance management mapping tool that was created by the project manager in FY2013, metrics and data for FY2016 were easily identified, which resulted in simplified mid-year and year-end evaluation processes. Brainstorming sessions were held with key staff members to identify DCR projects, initiatives, and activities to be considered for inclusion. Based on information gathered during the sessions, a matrix with options and recommendations was presented to DCR leadership, allowing for an informed decision-making and prioritization process.

To further support workforce alignment, the project manager assisted OPOS and OCRPRO office directors with aligning operational expectations for FY2016 with the respective branch/office strategy (which ultimately cascades to the U.S. Department of Health and Human Services' strategic plan) and collected associated data to report progress towards operational expectations.

The project manager also provided strategy and project management support to assist with the project close-out of Phidisa. The project manager facilitated final close-out meetings with the Phidisa lab and government-furnished equipment functional area groups to close out activities and transition specific project responsibilities to the South African Military Health Service. This effort was a huge undertaking as it is the first time DCR closed out a project of this magnitude. The project manager continued to track close-out progress after the project ended by updating both the Phidisa sub-study and close-out trackers as manuscripts were published and activities concluded. In addition, the project manager generated a milestone timeline with actual dates achieved, created an executive summary of the project for senior leadership, and assisted with developing a monograph with the Phidisa project team lead.

Learning and Professional Development

A Learning and Professional Development (L&PD) manager and a clinical training specialist provide learning and organizational/professional development support to the Office of Planning and Operations Support (OPOS) by serving as members of the L&PD Group. The three primary areas of support are: (1) identifying/developing training resources to address client-identified training needs; (2) providing training and professional development subject matter expertise; and (3) participating in professional development to ensure that staff members maintain their subject matter expertise.

The CMRP L&PD manager and clinical training specialist worked on a collaborative team, including representatives from the Office of Workforce Effectiveness and Resources (OWER) Collaborative Clinical Research Branch (CCRB) and the OPOS L&PD Clinical Research Oversight Manager (CROM), in reviewing the competencies identified for CCRB in the Human Capital Planning project. Based on its research, the team developed a new job function title and definition for collaborative clinical research scientific professionals, and several new scientific/ technical competencies were developed. An important outcome/use of the competencies identified for CCRB was a request for L&PD facilitation of, and participation in, training on "Crucial Conversations" specifically related to the communication competency that was identified as a high need for CCRB.

The L&PD manager served as the technical project manager and wrote a statement of work (SOW) to identify coaching resources, establish goals and the budget, and outline deliverables for coaches as part of the leadership development culture initiative in the Program Planning and Analysis Branch (PPAB). Once identified, the L&PD manager facilitated leadership and coaching support to the PPAB assistant branch chiefs, with the focus specifically on improving the dynamics of teams that are co-led.

The L&PD manager and clinical training specialist teamed with NIAID staff and Liberian clinical research staff on the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) Learning and Professional Development Program (LPDP) proposal. This focus of this effort is to work with key Liberian staff involved with NIAID research efforts to build capacity in the area of clinical research. The committee held virtual meetings each week from October 2015 to April 2016. The goals of the group included identifying key values, determining what success would look like for a Liberian LPDP (including functional job specifications), as well as determining categories of skills that need to be addressed and modes of training that can be made available to staff. The team identified risks that an LPDP would need to mitigate, and reviewed information collected from evaluations in order to identify the best types of trainings, education, and/or development opportunities to build clinical research capacity for Liberian clinical research staff. The committee then developed a proposal to be presented to the technical team at the annual PREVAIL

meeting. The L&PD manager wrote an SOW for The Mitchell Group's role in facilitating non-Ebola training initiatives using designated DCR funds.

The L&PD team participated with OPOS staff in the planning and logistics associated with preparations for two site managers who visited NIH/DCR/NIAID and Leidos from Liberia PREVAIL clinical research sites. The L&PD manager was a key participant in setting up the agenda for the Liberian visitors and also met with the visiting Liberian site managers, at their request, to explain how to set up a training program based on best practices.

Also related to PREVAIL, the L&PD clinical training specialist coordinated with key OPOS staff to provide PREVAIL pre-travel training for DCR volunteers traveling to West Africa; 75 people attended the 19 pre-travel training sessions that were offered. The training sessions were offered on a biweekly schedule through the end of October 2015. The L&PD manager coordinated a follow-up survey that was sent to all travelers who participated in the pre-travel training within a week of their return to the U.S. to determine the benefit of the training. The results of the survey showed the training was extremely helpful in preparing travelers for their assignments. At the end of this training effort, the L&PD manager compiled a lessons learned document and presented it to OPOS for full review.

The L&PD training manager is facilitating a review of the NIAID Data and Safety Monitoring Board (DSMB) training course to ensure it is accurate and consistent with the updated NIAID DSMB policy and supports the goal of the training, which is to provide an introduction to the purpose, objectives, organization, and responsibilities of a DSMB, as well as to statistical concepts leveraged in DSMB deliberations. The target audience is new DSMB members with minimal knowledge of DSMBs or individuals interested in obtaining a basic introduction to DSMBs. The L&PD manager and clinical training specialist reviewed the current DSMB computer-based training (CBT) and compared it to another CBT now available through the University of Wisconsin. The existing NIAID DSMB training was found to still be accurate and up to date. The comparison of the two trainings revealed a significant difference in length, which is primarily due to the extensive examples of DSMB activities that are included in the University of Wisconsin DSMB CBT. Both CBTs will remain available for training.

The L&PD manager was requested to participate in the NIAID Integrated Training Initiative and the NIAID Training Automation Sub-team in an effort to develop a site where NIAID staff can record the training sessions they take and find additional training opportunities related to their specific functions.

A training need was identified for PPAB to train the program specialists to write SOWs. The L&PD manager and the clinical training specialist worked together to provide this training.

The L&PD manager achieved her Myers-Briggs certification this year and offered four trainings on Myers-Briggs Type Indicator (MBTI) to the Intramural Clinical Management and Operations Branch (ICMOB).

The L&PD manager and clinical training specialist continue to support the NIAID Nursing Education Committee in the development, review, and marketing of the NIAID Nurses Orientation. This is a trans-NIAID initiative focused on providing consistent, accurate training materials for all nurses working at the Clinical Center, and it includes over 50 separate, competency-based training units and unit-specific evaluation tools. An annual review of selected topics is currently in progress.

Additional outcomes of this project include active participation in writing an article for publication in a nursing journal (to be selected) and writing the abstract for a poster presentation application at the International Association of Clinical Research Nurses, scheduled for October 2016 in Orlando, FL. The abstract was accepted for presentation at the conference.

The L&PD manager and clinical training specialist continued to work on the plan to award International Association of Continuing Education and Training (IACET) Continuing Education Units (CEUs) for the completion of each of the four courses of the NIAID Good Clinical Practices (GCP) Learning Center's online course. This plan involves adapting the current CBT to meet the IACET/American National Standards Institute quality standards. Once completed, anyone with a clinical research e-mail will be eligible to receive CEUs for this training.

Continued work efforts by L&PD are in process to complete a manuscript for publication of the Human Capital Planning process in a government setting. This will highlight the Human Capital Planning initiatives completed for several of our NIAID DCR customers.

The L&PD manager and clinical training specialist continue to support the Organizational Effectiveness Group (OEG), focusing, this year, on optimization and operational planning of strategy management, learning and professional development, special projects, knowledge management, and the expanding role of the deputy director of operations and management in DCR.

Technical Solutions Group

The Technical Solutions Group (TSG) within OPOS works closely with the Office of Cyber Infrastructure and Computational Biology (OCICB), Center for Information Technology,

the Office of the Director (OD) Property Office, and other partners to provide high-quality enterprise and innovative solutions to DCR offices and branches. A CMRP special projects administrator assists TSG with managing the technical, information, and data challenges encountered in a clinical research environment.

The special projects administrator supported the semi-annual Clinical Research Information Management System of NIAID (CRIMSON) contract award fee reviews to assess research subcontract performance against the metrics outlined in the SOW. Throughout each review period, the special projects administrator extracted data from monthly status reports and placed it into Microsoft Excel spreadsheets for comparative purposes; the special projects administrator also created dashboards for the review documentation packet used by panel members prior to the scheduled scoring/rating meetings.

The special projects administrator plays an integral role in the Acquisition Management and Operations Branch (AMOB) annual inventory of equipment, collaborating with the inventory team to reconcile property records and research the locations of missing and/or at-home equipment. In FY2016, more than 600 pieces of equipment were inventoried. The special projects administrator served as the central point of contact for ordering all technical equipment (e.g., laptops, desktops, monitors) upgrades and replacements, determined hardware and software specifications/requirements, coordinated specialty software purchases, and assisted in creating and managing the central computer annual budget, which is reconciled monthly with DCR financial reports.

The special projects administrator is responsible for external CRIMSON users, maintains their VPN and CRIMSON access, distributes security tokens, and keeps current records on accounts and returned tokens. Additionally, the special projects administrator has maintained responsibility for prioritizing, ordering, porting, and distributing phones and laptops for core full-time equivalent (FTE) employee travelers supporting the Ebola research program in Liberia.

The special projects administrator is the central point of contact for property information in DCR, helping users resolve any outstanding issues and liaising with the OD Property Office to coordinate property transfers, surplus, life cycle replacements, and property passes. In conjunction with the OD Property Office, the special projects administrator consistently maintains the NIAID goal of greater than the 80 percent of user verification of property among the users located at 5601 Fishers Lane.

Performance Measures

DCR is made up of seven branches and/or offices that provide critical support for expanding the clinical research enterprise. DCR facilitates state-of-the-art clinical research within NIAID and in global settings through: (1) oversight and management of intramural clinical research; (2) coordination of NIAID clinical research policy development and implementation; (3) regulatory monitoring and compliance; (4) statistical consultation; (5) operation of a state-of-the-art BSL-4 facility; and (6) capacity building in domestic and international settings. To build capacity in a domestic or international setting, it is important to develop the appropriate performance measures (i.e., metrics) that contribute to the mission and goals of the DCR and that capture the complexity and nuances of the interwoven, multifactorial, collaborative productivity that DCR's individual branches contribute to the facilitation of clinical research in other entities.

At the end of FY2013, Leidos Biomed established a research subcontract with Quality Science International (QSI) for providing advice and assistance to DCR for creating appropriate performance measures/metrics that provide data-driven, objective assessments of performance and productivity in NIAID's DCR. Ultimately, the data derived from the performance metrics may help DCR achieve better outcomes and higher productivity. The goals of the project are to: (1) develop and/or enhance key performance indicators (KPIs) for each branch within DCR; (2) identify comparison measures against which program performance can be compared; (3) employ a solid mixed-methods (quantitative and qualitative) approach to develop and validate performance metrics that can be used for quantifying performance, evaluating outcomes, capturing program process, and assessing quality; and (4) review potential dashboard systems and database features.

In January 2015, QSI dissolved and transferred the work under this subcontract to Jerry Lassa, Inc.; the same essential staff remained under the subcontract with Jerry Lassa, Inc. CMRP staff continue to manage this agreement, attending meetings, managing the budget, and reviewing progress.

During FY2016, the team from Jerry Lassa, Inc. worked with the NIAID Division of Clinical Research (DCR) to develop a strategy management process, and they completed tools to support the implementation of the strategy including an operations plan Excel template, work process improvement guidelines, example assessments, and examples of measures and targets. Mr. Lassa worked on defining strategy management process guidelines for OPOS, provided recommendations for the annual OPOS survey tool, and provided assistance to the Strategic Management Research Project Team (SMRPT) for continued review and scoring of the INA-RESPOND network using the Kaplan-Norton/ Baldrige approach defined by the SMRP team. The SMRPT is exploring what enhances or inhibits successful strategy management in clinical research networks. Mr. Lassa also

developed an interview guide and conducted a group interview with the INA-RESPOND consultants and summarized the findings. We expect work to be completed in FY2017.

Barriers to Clinical Research

CMRP provides support to DCR's Barriers to Clinical Research (BTCR) initiative with the maintenance and expansion of the web-based International Clinical Research Regulatory Matrix (ICRRM), also known as ClinRegs. CMRP has provided subcontract management and oversight to the ClinRegs website initiative through a research subcontract awarded to CSRA International (CSRA) in February 2014; there are four option years through September 2018.

The ClinRegs website contains clinical research regulations from around the globe, providing a database of country-specific information that allows users to explore regulations and compare requirements across countries. The website serves as a central resource for persons involved in planning and implementing international clinical research. ClinRegs also provides useful links to official regulations and other key resources—promoting an efficient and effective way to research clinical regulations. The following information is included on the website: competent authority oversight, ethics committee oversight, clinical trial lifecycle, sponsorship, informed consent, investigational products, and specimens.

During FY2016, CMRP staff members supported the following activities: ensured necessary communications were provided or directed to the proper stakeholders; maintained overall budget information; reviewed and approved invoices and monthly progress reports from the subcontractor; participated in teleconferences and meetings; and provided expert advice for operational risks to ensure adherence to scope, budget, and timeline. There are many weekly meetings between Leidos Biomed, the NIAID Project Officer, and the subcontractors. To effectively manage this project, two Leidos Biomed staff are needed to ensure coverage for all teleconferences and meetings.

NIAID and the ClinRegs working group finalized the country priority list for content to be added to the website in FY2017. The group analyzed the current and future countries where NIAID has been or will be conducting clinical trials and the overall budget for the projects. Using this information, the committee provided the list of priority countries, which was reviewed and approved by DCR's deputy director for clinical research and special projects.

During FY2016, the subcontractor developed content for and updated current content for 19 countries. CSRA researched regulatory information details via the internet and directly contacted resources within and beyond NIH to obtain and/or update applicable information. When necessary, this regulatory information is translated into English—a challenging aspect of the project has been obtaining reliable translations for the regulatory documents, which involves costly translation services. The team has relied on free web-based translations and sought assistance from individuals with appropriate language proficiencies who could critique or polish the translations—providing an invaluable service and cost savings. Prior to uploading information to the website, it is reviewed for both technical content and editorial accuracy.

In FY2016, CSRA led an effort to redesign the ClinRegs website to improve users' ease of maneuvering the site, navigation to the proper topic/subtopic to find information, and comparison between multiple countries. The team held multiple hands-on user acceptability meetings to determine what specific areas needed to be improved, and obtain suggestions on how the site could be improved as well as what additional information should also be included on the site. From this feedback, CSRA created suggested screen and website enhancements, which were discussed and decided on by the team. The new website was launched at the end of FY2016.

Office of Clinical Research Policy and Regulatory Operations

Regulatory Compliance and Human Subjects Protection Program

Support Provided by the Clinical Monitoring Research Program

CMRP plays a major role in developing and maintaining a regulatory environment that supports the National Institute of Allergy and Infectious Diseases (NIAID) intramural Division of Clinical Research (DCR) programs facilitated by the Office of Clinical Research Policy and Regulatory Operations (OCRPRO). The CMRP Regulatory Compliance and Human Subjects Protection Program (RCHSPP) provides support to key areas for OCRPRO with a Regulatory Affairs group, Clinical Trials Monitoring (CTM) team, Clinical Safety Office (CSO), Protocol Navigation/Protocol Development Program (PN/PDP), Learning and Professional Development (L&PD) group, as well as Document Control (DC) and Information Technology (IT) services. This infrastructure manages a portfolio of approximately 200 protocols.

The primary objective of RCHSPP is to maintain and enhance technical and programmatic support services for: (1) comprehensive clinical research management, regulatory support, and clinical safety oversight encompassing clinical trial monitoring and oversight; (2) Investigational New Drug (IND)/

Investigational Device Exemption (IDE)/Drug Master File (DMF) application development and management; (3) compliance with clinicaltrials.gov reporting requirements; (4) safety surveillance; (5) adverse event (AE) and safety reporting; (6) protocol and informed consent development and review; (7) investigational product oversight; (8) Data and Safety Monitoring Board (DSMB) and Safety Monitoring Committee (SMC) management; (9) protocol logistics and development services; (10) Institutional Review Board (IRB) support; (11) IT systems maintenance; (12) quality assurance (QA) compliance; (13) essential documents management; and (14) learning and professional development. All of these efforts are to ensure that NIAID-sponsored clinical protocols are conducted in accordance with U.S. Department of Health and Human Services (HHS), U.S. Food and Drug Administration (FDA), and National Institutes of Health (NIH) regulations and International Conference on Harmonization/Good Clinical Practices (ICH/GCP) guidelines. Additionally, RCHSPP oversees the establishment and maintenance of research subcontracts, provides logistical/project management domestically and internationally, and provides operational support to a variety of clinical projects.

RCHSPP efficiently and effectively supports the regulatory compliance, clinical monitoring, and safety surveillance aspects of clinical research, allowing principal investigators (PIs) greater opportunity to focus on the scientific objectives of mission-critical research protocols. More detailed information about the specific services provided to OCRPRO are described below.

Clinical Trials Monitoring

As an integral part of the RCHSPP, the Clinical Trials Monitoring (CTM) team facilitates and oversees NIAID/OCRPRO-supported cutting-edge clinical research for Phase I and II IND/IDE and non-IND intramural trials.

The overarching responsibilities of the CTM team include monitoring studies to ensure that the rights, safety, and well-being of human subjects are protected; verifying source documentation; ensuring the accuracy and completeness of reported study data; ensuring that study conduct complies with the protocol approved by the IRB/ethics committee, ICH/GCP guidelines, and all other applicable regulatory requirements; detecting, reporting, and assisting with site quality management planning; resolving discrepancies that occur during the study period; and communicating all site-monitoring reviews and observations to PIs and clinical research oversight managers. The team also ensures that the sites maintain study agent(s) and devices that are in compliance with study protocols and FDA regulations for an IND or IDE.

CTM staff consists of a clinical trials director, an associate clinical trials director, 4 clinical trials/ project managers, a clinical database project manager, 10 clinical research associates, a clinical data analyst II, and a program coordinator. There are currently three open positions. The team's main focus is to facilitate and oversee clinical research studies.

Currently, CTM is involved with managing and monitoring approximately 194 active NIAID clinical research studies conducted at sites throughout the U.S. and in several foreign countries. The types of studies vary and include Phase I/II IND and IDE studies, natural history studies, studies involving pediatric subjects, and research studies that are minimally to noninvasive and do not fall under an IND/IDE.

During FY2016, the team conducted more than 5 pre-study site assessment visits, 63 study initiation visits, 248 interim monitoring visits, 3 audit visits, and 55 study close-out visits. Team designees conducted two protocol audit visits for the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) study. Clinical trials monitoring was conducted at various international clinical sites across the world, including those in Argentina, Bangladesh, Cambodia, Cameroon, China, India, Mali, Sierra Leone, Thailand, Uganda, Vietnam, Liberia, Mexico, and South Africa. The CTM team and designees also conducted audits and study start-up site visits at locations in Argentina, Liberia, Mali, Mexico, and South Africa.

NIAID Networks

The CTM team continues to provide sponsor-related clinical trials management for several established NIAID networks, including the Influenza Research Collaboration (IRC) Network, an Intravenous Immunoglobulin (IVIG) study, the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) network (closed in winter 2016), South East Asia (SEA) (which ended in FY2016), and the Mexico Flu networks. The team also continues to support studies in the Washington, D.C. area that are part of the District of Columbia Partnership for HIV-AIDS Progress (DC-PFAP) program (which ended in FY2016) and the Department of Defense Infectious Disease Clinical Research Program (IDCRP).

Mexico La Red Network

The CTM team has been involved with the monitoring aspects of studies conducted under Mexico's Emerging Infectious Disease Clinical Research Network (La Red) since January 2010.

There are six sites within the network; however, currently, five of the study sites are actively recruiting subjects. Based on enrollment, the CTM team conducted on-site monitoring for four of these sites, provided close-out procedures for one site, and performed an initiation visit for the last site. In November 2015, CTM assisted the Network Coordinating Center (NCC) on a site assessment visit for a potential site in Oaxaca, Mexico. During the fall 2015 La Red Annual Meeting, CTM presented monitoring visit findings and lessons learned to the attendees. In early 2016, the local (Mexico) clinical research associate (CRA) resigned from the NCC. Therefore, the NCC currently does not have a CRA to perform the monitoring responsibilities and is working to fill this position. In an effort to support our customer in the continuance of monitoring the current work flow, CTM proposed to subcontract a U.S. outside vendor to assist with the monitoring aspects until an appropriate candidate is hired. This plan was agreed upon by the NCC director and is being processed. Once executed, CTM will be involved in training, facilitating the monitoring tasks of the contracted CRA, and reviewing/approving monitoring reports/letters.

CTM designees assisted with preparing a presentation that the CTM clinical research oversight manager will give during a training meeting for La Red's new Zika study to be conducted at three to four new sites.

Influenza Research Collaboration Network

For the IRC Network, the CTM team manages the sponsor's essential document files, as required by the FDA and HHS, for more than 62 domestic participating sites, plus 20 international participating sites, including Argentina, Thailand, and Mexico, across the NIAID Influenza Research Collaboration (NIRC) and INSIGHT, and conducts sponsor-site audits. During FY2016, the CTM team, in conjunction with a contracted local U.S. clinical research organization, expedited the initiation of 37 close-out visits. Cost-saving measures established last fiscal year, which continued into FY2016, eliminated some annual site-monitoring visits for IRC study sites that did not have any subjects randomized. This year, several IRC network protocol study visits were combined in order to cut costs.

As another cost-saving measure, close-out visits for IRC 003 sites that did not enroll any subjects were conducted via teleconference. During FY2016, approximately 15 IRC 003 close-out visits and 10 IRC 005 study-initiation visits were conducted in this manner, eliminating the need for costly travel.

A clinical project manager reviewed topics, including study agent accountability and request, monitoring, and lessons learned for the investigators' meetings held for the IRC 003 and IRC 005 studies in December 2015.

Due to an unexpected issue with the study agent vendor during the influenza season (FY2016), the study agent supply in the repository was exhausted for the IRC 003 study in the U.S. A clinical project manager worked with the clinical trials director and a CMRP contracts clinical project manager to develop a process for transferring the study agent from Argentina sites that were closing and poor-performing U.S. sites to a rapidly enrolling site in the U.S. that was able to store a large volume of the study agent on site. The process was approved by OCRPRO, and the transfer occurred, saving the enrollment for the successful influenza season.

During the 2015–2016 influenza season, the study agent required stability retesting prior to the approval of continued use. A clinical project manager and the clinical trials director worked with members of the IRC 003 protocol team to ensure that appropriate retesting labels were distributed and all affected sites were notified. The clinical project manager developed memos to inform the study teams of the changes, worked with the site management vendor, Social and Scientific Systems, Inc. (SSS), to develop and distribute instructions for relabeling, and ensured the completion of processes before allowing sites to enroll any additional subjects or distribute the study agent.

The study agent was depleted in Thailand for the IRC 004 protocol during the 2015–2016 season. To address this issue, a clinical project manager worked with the clinical trials director and a CMRP contracts clinical project manager to develop a process for transferring the study agent from Argentina to Thailand. The study agent was transferred and then able to be provided to the rapidly enrolling infectious disease site. This transfer process included the immediate shipment of the new study agent supply to Thailand once it was produced, avoiding any delays in site enrollment.

Following the end of subject enrollment of the IRC 003 study in April 2016, CTM facilitated the monitoring of the necessary subjects/sites in order to meet the study monitoring plan deadline of July 2016. This aggressive timeline for final analysis and study closure ensured that all sites opened during influenza season 2015–2016 for IRC 003 were able to be considered for analysis appropriately prior to final database closure.

Southeast Asia 50 Study

The CTM team continues to provide oversight of Pharmaceutical Product Development (PPD), Inc. for monitoring functions that are carried out in Thailand, Vietnam, Bangladesh, and Korea. The 11 sites in the Southeast Asia 50 study (SEA050) have completed their enrollment and closed the study at their institutions, and PPD has monitored and closed the protocol accordingly. CTM worked closely with the project manager and PPD in order to meet the budget timelines.

A new dengue study based on clinical trials conducted at the Johns Hopkins Center for Immunization Research began in Bangkok, Thailand in late FY2014, and continues to be monitored by PPD during FY2016. In addition, a sister dengue study began in Bangladesh, and PPD will monitor this study twice in FY2016. A study in China that utilized PPD monitoring closed in early FY2016. The immune reconstitution inflammatory syndrome study being conducted at two sites across Thailand has continued and will close by the end of FY2016. Dr. Christa Zerbe's (Laboratory of Clinical Infectious Diseases, NIAID) study has also continued, with annual monitoring at one Thailand site. A new study involving several sites in South Africa and one in China has been delayed and is now slated to begin in late FY2016. CTM staff will continue to work with NIH staff and the PPD clinical research organization to ensure that additional studies are executed in a timely manner and within all applicable guidelines.

Africa

The contracted CRA, stationed in Benin, West Africa, travels to Mali and continues to provide clinical trials monitoring support. This CRA works closely with CMRP staff to ensure that the NIAID clinical trials are effectively monitored and helps to ensure that the rights, safety, and well-being of human subjects are protected. This CRA presents GCP and regulatory training to Malian study teams, with typically more than 30 staff members attending the training sessions that include instruction on adequate source documentation, proper informed consent form completion, AE/serious adverse event (SAE) reporting, and other pertinent clinical research conduct topics. Based on the success of previous trainings, the CRA is often asked to return and present new information or similar training materials to new staff. As an additional service to our customers, this CRA became a certified trainer who can issue continuing education units (CEUs) to participants.

Two IND studies and several natural history studies are currently monitored in Mali by this CRA. The CRA provides firsthand accounts of these ongoing clinical trials, which are reviewed with the Malian investigators and also reported back to the NIAID-affiliated investigators. Effectively communicating the positive findings and areas for improvement to the site staff and NIAID investigators is critical to the success of the trials conducted there. The CRA's review of these studies helps ensure the reported study data are accurate, complete, and verifiable from source documents; ensures that the study conduct is in compliance with the protocol, ICH/GCP guidelines, and applicable regulations and standards; and detects, reports, and resolves discrepancies that occur during the conduct of the study.

This CRA closely reviews recorded trial data, understands site processes, and continually reviews the protocol so that the protocol-intended language written by the NIAID investigators adequately reflects what can realistically and logistically occur on the ground in Mali. Recently, the CRA raised the question of whether the Malian investigators have all the tools to adequately perform body system reviews at the level expected by the NIAID investigators. Noting there might be a difference between the two expectations and communicating this to the parties involved helped ensure a cohesive understanding of the protocol requirements, and lessons learned from this experience can help standardize the definition of a physical exam for future studies conducted in Mali and possibly elsewhere.

The CRA's findings often influence procedural processes that are assessed and improved upon to fulfill the needs of subjects, study staff, protocol requirements, and regulatory and IRB/ethics committee requirements. Standard operating procedures have been written and continue to be revised based on lessons learned from this CRA.

This CRA has also assisted with the start-up and monitoring of a new study being conducted in Cape Town, South Africa. A site-initiation visit was conducted at the TASK Applied Science site, and the CRA will conduct biannual monitoring visits at the site.

Johns Hopkins University

During this reporting period, a multitude of trials continue to be run at the Johns Hopkins University Center for Immunization Research (JHU CIR). The trials include respiratory syncytial virus (RSV), influenza, West Nile virus, Ebola, and several related to the dengue virus. Many of the studies are also being run at the University of Vermont (UVM) site. Three new studies have started during this reporting period: one using both the JHU CIR and UVM sites and two run at the JHU CIR site. Two studies will have site-initiation visits later in FY2016. The CTM team has supported the site-initiation visits and continues to monitor these protocols along with several ongoing trials at the sites.

The Laboratory of Infectious Diseases (LID) has also continued to conduct studies at the University of Rochester Medical Center (URMC). Two new studies are slated to begin at URMC during the reporting period, and one study had a site-initiation visit completed but was unable to start due to seasonal flu restrictions. The principal investigator/site plan to have more studies active in early FY2017. One study is still open at the site and continues to be monitored.

Department of Defense Infectious Diseases Clinical Research Program

One new multicenter study was planned to be initiated in FY2015 (Department of Defense [DoD] Infectious Diseases Clinical Research Program

[IDCRP]-080 [Prevent TD]); however, due to delays in the study agent availability that DoD encountered, the site-initiation visits were postponed. The CTM team conducted three site-initiation visits for this study in November 2015, and, as a cost-saving measure, two of the sites joined via teleconference. In February and May 2016, two additional site-initiation visits were conducted, one of which was at a site just added to the protocol. This site-initiation visit was conducted on an accelerated time line in order to meet the customer's needs.

Additionally, CTM is providing ongoing guidance to a multinational study conducted by IDCRP (TreatTD). The CTM team continues to review all protocol amendments that affect the activated DoD/IDCRP general infectious disease and HIV studies monitored by CTM.

RCHSPP worked to close all IDCRP-045-01 (FluPro) study sites and transition the IDCRP-037 (TravMil) study to IDCRP for quality assurance/ quality control procedures instead of monitoring since IDCRP-045-01 will no longer enroll pediatric subjects. In FY2016, four of the five participating sites were transitioned. However, two sites were added to this protocol, and monitoring will continue until the monitoring plan has been met at these sites. CTM will continue to review protocol amendments and quality assurance/quality control reports to provide guidance. This transition will be a cost savings.

During a recent monitoring visit at one of the DoD sites, significant issues with source documentation were identified. CTM worked with the IDCRP deputy science director to inform site officials of the findings prior to publication of the site visit letter. This collaboration was noted positively by the director. CTM is working to complete the monitoring of this study in FY2016.

D.C. Partnership for HIV/AIDS Progress

The CTM team worked closely with investigators to support the closure of studies in the Washington, D.C. area that are part of the D.C. Partnership for HIV/AIDS Progress (DC-PFAP) program, specifically, the CONQUER study (Safety, Tolerability, and Efficacy of Asunaprevir and Daclatasvir in Subjects Coinfected with HIV-HCV) was closed on an expedited time line. The CRA worked with the site principal investigator and staff to meet their needs.

Protocol and Informed Consent Reviews

The CTM team reviewed clinical research protocols and informed consent forms, providing commentary to NIAID, IDCRP, and principal investigators at JHU, UVM, and the University of Rochester. The group also reviewed and revised IDCRP protocols, source documents, and case report forms (CRFs) on new studies (IRC 005).

Overall, CTM worked with other RCHSPP functional teams to review approximately 25 initial clinical research protocols/informed consent forms, 85 amendment reviews, 5 site-specific informed consent forms, and 6 navigational initial reviews during this reporting period.

Designees from CTM are continuing to work with the Clinical Research Information Management System of NIAID (CRIMSON) staff and the OCRPRO clinical research oversight manager to develop CRIMSON to allow electronic monitoring of NIAID studies. Electronic monitoring will reduce the need for the database reports supplied by study coordinators, saving time and reducing overall costs to our customer. Members of the CTM team continue to meet with the CRIMSON staff on a monthly basis to work on this project. Several demonstrations of the electronic monitoring function have been given, and CTM has provided advice for developing this new platform and has reviewed several guidance documents to help with this process. In FY2016, a working group was formed that will test the monitoring in the system at one of the sites. The working group is hoping to implement this application at all sites using CRIMSON in FY2017.

Data Systems Support

The CTM team continued to utilize FrameMaker/ DataFax to create electronic CRFs. Capabilities for providing remote data monitoring will be further investigated during FY2016. The creation of electronic CRFs using FrameMaker/DataFax continues to be effective. The CTM team has worked on approximately seven new CRFs and has modified 18 CRFs this fiscal year. Several steps are included in the creation of CRFs, and, after finalization of the forms, the database setup can often take several weeks. Some of the CRFs are utilized on tablets as a method of data capture. This approach takes more time for set up in the database, but eases the data capture process. A new protocol in Cameroon is slated to utilize this approach for data capture.

DataFax Database line listings have been provided to CRAs for their use during monitoring visits. These line listings are generated and provided by the Office of Cyber Infrastructure and Computational Biology (OCICB) team prior to monitoring visits. The CRA will then compare them to the source documents during their site visits. This process allows for the comparison of the data to be analyzed to the source documents at the site.

A clinical project manager and a clinical data analyst created a questionnaire for investigators regarding the FrameMaker CRFs that we provide for data collection. The survey is automatically provided to investigators shortly after the CRF packets are completed, allowing the team to receive feedback in a more timely manner. Responses from the investigators

regarding the completion of FrameMaker CRF packets indicated general satisfaction, in addition to noting areas for improvement.

CTM is in the process of contracting a local U.S. clinical research organization to fulfill the monitoring responsibilities at the NCC, while the vacant CRA position for the Network Office in-country is being filled. Once executed, CTM will be involved in training, facilitating task monitoring of the contracted CRA, and reviewing/approving monitoring visit reports and letters.

Ebola Clinical Research

In response to the expansion of the Ebola research efforts, with new studies and plans for HIV studies in Liberia, RCHSPP CTM hired a CRA III in March 2016 and a CRA II in June 2016. Both CRAs will travel to Liberia and Sierra Leone regularly to monitor the two ongoing and two new Ebola studies. The CRA III lives in the U.S. and can assist with monitoring other studies as needed. The CRA II is based in Senegal, Africa, which is anticipated to be a cost savings on travel.

A clinical project manager, clinical research associates, and the clinical trials director developed a small Ebola working group. The CTM working group provided support via regulatory documents, expedited site activations, development of QA processes, development of flow sheets for clinical staff, and guidance for the teams in Sierra Leone and Liberia. The CTM work group also developed site training for remote locations and assisted in the training of Liberian staff.

CTM has assisted in the training of local clinical research staff in GCP and applicable FDA regulations. In May 2016, two Liberian site staff met with the clinical trials director and associate clinical trials director to review findings from a recent monitoring visit and review specific GCP. The meeting was productive and the staff requested additional training.

In FY2016, CTM finalized a guidance document on the appropriate delegation of responsibilities for investigators and site staff.

Document Control

The Document Control (DC) group is at the center of RCHSPP's quality system as it maintains critical contractual and regulatory documents for all open protocols, and archives documents associated with closed protocols. DC offers the following services to assist the various RCHSPP groups: (1) establish and maintain RCHSPP standard operating procedures (SOPs); (2) establish and maintain CMRP standard procedures (SPs); (3) process annual reviews of policies, SOPs, reference guides, SPs, and associated forms; (4) scan and archive documents; (5) train staff on the DC system and the various electronic documents maintained within the system; (6) assign project codes; (7) oversee the off-site archiving and retrieval of documents stored at the Iron Mountain records storage facility; (8) generate audit reports, and audit regulatory and administrative documents; (9) generate and manage version-controlled documents; (10) establish procedures for issuing, tracking, and reconciling all documentation; (11) maintain locked drives, the shared drive (which houses current protocol documents, completed protocols, and current and historical curriculum vitaes [CVs]), the regulatory drive (contains locked IND folders), and the clinical safety drive; (12) develop and maintain secure filing systems for all hard and electronic documentation; (13) initiate and track RCHSPP protocol reviews; and (14) create CDs, various tracking tools, and logs.

During the year, DC managed files for active protocols and active INDs/IDEs/DMFs, assigned new project codes, and processed regulatory submissions, regulatory annual reports, non-FDA submissions, non-FDA correspondence documents, regulatory correspondence documents, and FDA meeting minutes. The DC group also processed regulatory electronic Common Technical Document (eCTD) submissions, regulatory master file (MF) submissions, IDE regulatory submissions, processed and stored DSMB randomization codes for the Clinical Safety Office (CSO), processed DSMB summary reports and DSMB Conflict of Interest Forms, as well as Safety Monitoring Committee (SMC) meeting minutes and SMC Conflict of Interest Forms.

DC also processed Independent Medical Monitor (IMM) Meeting requests and IMM Conflict of Interest (COI) requests, posted Package Inserts to the CMRP shared drive, and managed bucket requests.

There are currently more than 550 boxes, equaling approximately 665 cubic feet of documents, stored at Iron Mountain, a contracted off-site storage facility. The DC group oversees the archiving process and manages document retrieval requests.

DC scanned SAEs for CSO, processed new document requests, initial reviews, final reviews, final directorate reviews, and signature cycles of policies, SOPs, reference guides, SPs, and associated forms. In addition, DC completed annual reviews of SOPs, forms, policies, reference guides, SPs, and related forms

During the year, DC managed files for more than 190 active procedures, including SOP revisions and reviews, processed signature approvals, and managed completed workflows in the SOP management system.

Institutional Review Board Support

The RCHSPP Institutional Review Board (IRB) administrator provides administrative and programmatic support to NIAID's IRB. Working in collaboration with the Office of Clinical Research

Policy and Regulatory Operations (OCRPRO), the IRB administrator efficiently and effectively processes documents for IRB submission. Support efforts include acting as a liaison between the study teams and the IRB, including regular study coordinator update presentations; processing full board and expedited protocol actions for review; preparing meeting packages; tracking protocol submissions from initial submission through the Office of Protocol Services (OPS) approval phase; preparing tracking reports; and maintaining protocol-specific records. These efforts facilitate the conduct of research within the NIH Intramural Research Program.

The IRB administrator provided comprehensive support by processing incoming submissions and submission approvals, including reviewing submission components, identifying deficiencies, and providing administrative stipulations and guidance to investigators; processing final approvals from OPS; updating the manual logs of protocol submissions and renewals; providing advice to investigators and study staff; contributing to procedure discussions regarding new and/or changing NIH policies that affect the NIAID IRB; preparing IRB meeting packets; preparing electronic meeting packets for IRB members who prefer to review documents online; and attending IRB meetings in person throughout the year.

The IRB administrator provided support to special projects, including the utilization of iMedRIS (iRIS) web-based IRB submission software by serving on the iRIS development work group. The IRB administrator worked with IRB staff and the iRIS trainer to identify and correct issues with the updated iRIS applications that were impacting ease of use. These efforts support OCRPRO's strategic priorities by directly aligning to the OCRPRO goal to "evaluate services and solutions and apply process changes as needed" specific to the objective and "develop policies, guidance, documents and processes consistent with the spirit and intent of federal requirements that are focused on facilitating effectiveness of the research enterprise." The IRB administrator also conducted a training on the iRIS system for staff from the Research Oversight and Compliance Division, Air Force Medical Support Agency in January 2016. These individuals reached out to the NCI and NIAID IRBs to obtain guidance on a strategy for effective use of the iRIS system. The IRB administrator provided a tutorial of creating submissions, obtaining sign off, and reviewing signatures in iRIS, as well as common problems that have arisen with the NIAID iRIS system.

In addition, the IRB administrator assisted in implementing updated SOPs as a part of NIH's Association for Accreditation of Human Research Protection Programs (AAHRPP) certification, presented quarterly trainings to keep study coordinators informed on IRB activities and changes to NIH policies, and ensured that the NIAID IRB website remained up to date.

During FY2016, the IRB administrator took on new projects related to knowledge sharing and dissemination of information. These included coordinating study coordinator updates, as well as conducting one-on-one presentations for new study coordinators on topics such as NIH policies on conflicts of interest, the emergency IND application process, and the AAHRPP re-accreditation process.

The IRB administrator reviewed the Continuing Review, Amendment, Safety Reporting, and External Resources sections of the NIAID IRB website, and coordinated the necessary tasks to update these components of the site. This involved communication and coordination among the NIAID IRB Office, OCICB, and NIAID IT.

At the March 2016 NIH IRB Professional Administrators Committee (IPAC) meeting, individuals from two other NIH institutes requested guidance on streamlining IRB submission review through the use of standardized checklists. The RCHSPP IRB administrator sent a collection of checklists and process guides used by the NIAID IRB office with the goal of promoting a consistently high level review of intramural research across institutes.

Information Technology

The RCHSPP Information Technology (IT) group provides software development, computer, network, application, and backup/disaster recovery support services for NIAID initiatives. Staff members include 1 IT manager, 3 programmer analysts, 2 systems administrators, 1 network specialist, and 1 secretary.

Design, development, testing, and implementation of over 40 new TrackWise system service and product requests were completed in FY2016 and a new monitoring visit report was developed to better assess project workloads for clinical monitoring staff members.

More data is being captured to track halted protocols and an associated report has been developed. The data captured to track these protocols include many logistical and reporting timelines and dates to ensure that if and when a protocol is halted, all appropriate staff and OCRPRO personnel are notified. Halted protocols are occurring more frequently and the Clinical Safety Office (CSO) requested this report to track instances and provide detailed updates to customers.

Because the CSO is also responsible for managing and facilitating DSMB, SMC, and Independent Safety Monitor (ISM) meetings, new data elements are being tracked and a new safety oversight report was developed to assist with meeting logistics. The information was formerly tracked manually but can now be delivered electronically through TrackWise.

The regulatory process of having multiple groups perform an initial review for the IND Annual Report submitted to the FDA has been formalized and implemented as part of the regulatory document review process within TrackWise and is now more adequately tracked. Previously, initial review requests were conducted via telephone and email.

The TrackWise platform itself was enhanced during FY2016. Upon learning of an upcoming initiative by NIAID OCICB to deploy the Internet Explorer 11 browser to all user workstations, RCHSPP IT staff quickly procured, tested, and implemented the latest version of software for the TrackWise production, development, and testing environments. The implementation path to upgrade these environments to version 8.7.10 included performing configuration modification, testing, and validation exercises, and modification and deployment of Crystal Reports templates. The upgrade not only provided the necessary support for the Internet Explorer 11 browser, but also provided support of the Microsoft Windows Server 2012 R2 and Microsoft Office 2013 platforms. Functionality changes were minor with the exception of one: The execution of queries and running reports can now occur simultaneously in separate accessible windows, thereby improving usability and efficiency for the end user.

Obtaining customer feedback is an essential aspect of providing high-quality customer service. This year the IT group was requested to develop three surveys to allow our government customer to collect data for the enhancement of their service offerings. The NIAID Orientation Topic Feedback Survey and the NIAID Nursing Orientation Survey solicited input on the NIAID Nursing Onboarding program to allow fine tuning of this new program by participants while the DSMB Satisfaction Survey provided insight into the current process flow for conducting DSMB meetings. Two additional surveys were developed to seek input from clinical trial study staff. The first was created to seek feedback from the study sites and investigators regarding their experience with the RCHSPP CRF process with the Clinical Trials Management group. The second survey was developed and is distributed during study closure to evaluate how well the CRFs met the needs of the study staff for the duration of the study.

The IT group provided ongoing technical support to the eCTD system for the submission of regulatory documents to the FDA through the FDA's electronic submission gateway. Ongoing support of the eCTD publishing system included the installation of several product service releases, reallocation of the publishing software from two dedicated kiosks to individual Regulatory Affairs (RA) staff members' workstations, and timely responses to support inquiries from the RCHSPP RA group for the Omnicia eCTD publishing system and Verisign digital ID certificate, which are used for submissions to the FDA through the electronic gateway. The IT group also continues to serve as technical liaison for the RA group to ensure that software interoperability exists with United States Government Configuration Baseline (USGCB) group policies and security updates.

Ongoing core IT functions provided to CMRP span a broad spectrum of technologies and service offerings, including: (1) the application of whole-disk encryption to all new laptop computers, encryption key recovery services, and audits to ensure continued compliance with the Office of Management and Budget (OMB)/HHS directive for protection of sensitive information; (2) the evaluation, specification, acquisition, integration, and management of computer hardware/software; (3) system administration, technical support, and backup/disaster recovery services for program staff in both domestic and international settings; (4) the standardization of government-furnished Microsoft Windows personal computers in compliance with the USGCB mandate via technical analysis and review of federal policies and procedures, establishment of project plans, analysis of software impact, dissemination of communications to program staff, categorization of resources into applicable security containers, development and submission of waivers, and generation and allocation of secondary administrative accounts; (5) the installation and monitoring of McAfee ePolicy Orchestrator for the management of site antivirus and related security software; (6) the collection, evaluation, design, and implementation of change requests for TrackWise, the quality and process tracking system for the program; (7) the development, unit testing, and maintenance of custom Crystal Reports for correlative analysis, qualitative and quantitative process/data measurements, and end-of-month/quarter/year summaries from TrackWise; (8) participation in RCHSPP strategic planning sessions, working groups for Section 508 compliance, TrackWise, and Electronic Document and Records Management System (EDRMS), as well as FDA inspection readiness teams; (9) the evaluation, procurement, and deployment of encrypted Universal Serial Bus (USB) keychains to staff in adherence with HHS policies; (10) the development of IT training materials and presenting at New Employee Orientation (NEO) sessions; (11) the provision of management, maintenance, and support services to the core site network and data services infrastructure; (12) the design, development, hosting, integration, and maintenance of Microsoft SharePoint Services platforms; (13) participation as a member of and key contributor to several technology-related project teams, including the Leidos Biomedical Technology Review and Advisory Committee, Microsoft Active Directory Working Group, Health Information Portability and Accountability Act (HIPAA)/Health

Information Technology for Economic and Clinical Health (HITECH) Act committee, and CMRP Leadership Advisory Group; (14) provision of video conferencing and video collaboration support services for both near and remote locations; and (15) provision of services to ensure compliance with smart card authentication requirements and standards set forth by the Homeland Security Presidential Directive 12 (HSPD-12) Act of 2004 and associated Federal Information Security Management Act regulations, OMB memoranda, and NIH policy.

The IT group, in conjunction with the CMRP L&PD group, continues to support the deployment of TrackWise Training Manager for tracking and managing training records for every program employee, from noncurricular group training to individualized curricular training. To date, more than 14,300 noncurricular and 7,400 curricular records have been entered and managed through the system. Recent curricular trainings deployed include the 2016 versions of Information Security Awareness and Privacy Awareness. For staff members who travel to high-risk areas, a mechanism to capture a new training called the High-Threat Security Overseas Seminar has been implemented.

The integration of the OpenText Enterprise Content Management suite, formerly known as Livelink and also known as EDRMS, and TrackWise to manage content for clinical protocols undergoing an initial or amendment review by RCHSPP continues to be a successful blend of two systems and is used extensively within the program.

To help mitigate the risk of data loss due to fluctuations in power, the IT group has deployed and supported the use of uninterruptible power supply (UPS) units to computer workstations. In the event of a power outage to the site, the units will support the operation of the computer to provide sufficient time for critical data to be saved. As batteries are a consumable part, the IT group has actively replaced the units deployed in prior years to ensure all workstations at the site are properly protected.

In collaboration with NIAID OCICB, the IT group was successful in upgrading the Microsoft Office 2010 suite to the Microsoft Office 2013 suite on all workstations. This upgrade was a required step in upgrading all work stations to Microsoft Office 2016, which will in the very near future and is required to comply with HHS directives.

In order to make space available in the five network closets in preparation for the replacement of all Industry Lane phone systems with a Cisco Voice over IP (VoIP) platform, the IT group worked closely with the NIAID OCICB network team to identify and relocate or replace network equipment. Some of the devices had been in operation for several years and, if replacement was not performed properly, network service interruption to the site could have occurred. To minimize the potential for disruption, equipment acquisition and configuration was performed in advance and the installation activities were scheduled during non-working hours. As a result, new networking equipment was successfully installed, remaining equipment was relocated, and all equipment was verified for proper operations, with network operations resuming as expected prior to the start of the next business day.

To accommodate a rapidly changing and mobile workforce, the IT group leveraged available software solutions to support teleworking and remote collaboration. However, the array of tools available and lack of availability of materials that adequately describe the use of these technologies resulted in frequent inquiries and technical support requests by end users. To best satisfy this new environment, the IT group developed CMRP Remote Collaboration Technologies training materials that team members presented to CMRP staff to introduce them and guide them on the use of the many available collaborative technologies including Citrix XenApp/XenDesktop, Citrix GoToMeeting, Cisco VPN, and Skype for Business. This material was well-received and continues to be provided to new hires during new employee orientation.

The IT group worked closely with members of NIAID OCICB to develop, test, and implement a new vulnerability management system that scans and reports any security vulnerabilities found on NIAID workstations that would require some form of remediation. In order to determine the best strategy for implementation, a stakeholder team comprised of team members within the IT group and NIAID OCICB was formed, with reoccurring biweekly meetings scheduled to promote sharing and discussion. As a result of the collaboration and participation by the RCHSPP IT staff, NIAID OCICB was able to successfully deploy the new vulnerability management system across the institute.

Learning and Professional Development

L&PD support for RCHSPP/ OCRPRO is provided by a clinical training manager, 2 clinical training specialists, and an administrative support staff member. The primary area of support is to provide quality continuing education and training programs to address regulatory, technical, and professional skills competency, and encourage professional development by identifying and facilitating training events for our diverse customer base.

L&PD is active in the TrackWise Working Group, collaborating with the IT group to optimize the configuration of TrackWise Training Manager for RCHSPP training records management.

L&PD continues to offer courses that are eligible for the International Association for Continuing Education and Training CEUs, while expanding processes to include training sessions provided by third-party vendors and access to “durable training materials.”

L&PD renewed its subscription of the FDANews webinar training pass, which allows our clinical groups to view past, current, and future webinars for one fixed price instead of paying a per-webinar fee.

L&PD has developed a partnership with the clinical trials subcontractor to offer CEUs for the GCP training provided in Mali. The first session of the course was offered in April 2016. Forty-seven people participated in this first session. L&PD worked closely with the subject matter expert (SME)to receive the training assessments and evaluations, and to develop a plan for providing feedback to each participant. Of the 47 attendees, 31 participants received CEUs.

The L&PD group continued to maintain a spreadsheet identifying FDA Warning Letters citing GCP issues; this spreadsheet is utilized extensively by clinical research professionals to ensure compliance.

During FY2016, the L&PD group facilitated trainings and webinars on various training topics, including Creating Visual Presentations, Becoming a Great Clinical Monitor—Monitoring with an Auditor's Eye, Recent Trends in Noncompliance and Safety Assessment for IND Safety Reporting, and Issues with Informed Consent.

The clinical training manager developed and presented courses to our core clinical groups that included Professionalism in the Workplace and Communication's Impact on IT, Communication Style Preferences, and Introduction to the Myers-Briggs Type Indicator (MBTI).

L&PD facilitated the following training opportunities: a two-day Clinical Trials Management training provided by an outside vendor and a three-hour webinar on a software (FrameMaker) that enables our Clinical Trials Management staff to design CRFs.

L&PD worked closely with the senior management of the Clinical Trials Monitoring group to identify an outside vendor to customize in-depth training for new staff. The two-day training, titled Clinical Trial Management, included an overview of Good Clinical Practices and human subjects protection to set the stage for how regulations evolve; identified key codes of conduct, and reviewed requirements for informed consent process and supporting documentation expectations; included clinical trial fundamentals, and provided a high-level overview of study/device classifications, source documentation, informed consent forms, essential documents, etc.; included Clinical Monitoring and Reportable Events review source document information and reviewed case studies. CEUs were offered for this course.

To support new staff arriving in the Clinical Trials Monitoring group, an outside vendor was contracted to provide a three-hour webinar on the software FrameMaker, which is used to create CRFs in support of the OCRPRO principal investigators who use CRFs internationally.

Project Management

The RCHSPP Project Management Team (PMT) continues to provide strategic and operational planning, and project management, reporting, and logistical support services to enhance the capacity of the NIAID OCRPRO in conducting its mission and maintaining the infrastructure needed for RCHSPP to fulfill program management and contractual requirements. In collaboration with all RCHSPP program support team members and functional groups, PMT works to align organizational strategy and operational insight into project and program initiatives with the tactical goals and objectives required to achieve overall success within RCHSPP.

The main operational and strategic focus is to provide insight for managing projects/program success at the appropriate level of the RCHSPP/ OCRPRO to cover a myriad of program planning, resource forecasting, tracking, and reporting requirements as specified by the customer.

At the end of FY2015, the PMT continued to update program baseline reports that were used to measure how overall program resource use and portfolio status aligns with the original baseline plan. The comparison of these two program baselines was aimed at providing key managerial and operational insights for senior managers in terms of overall resource utilization and requirements by all functions, research groups, and protocols. This comparison also enables senior management to respond proactively in making necessary adjustments to overall budgetary and resource requirements.

Using historical data, including from the end of FY2015, the PMT continues to define and refine the three major internal benchmark indicators (metrics) that enable senior managers to assess overall resource use and cost savings through resource-capacity planning and optimization within RCHSPP. These three key metrics include unit cost of research support, resource use rate, and protocol growth. These metrics are still at the preliminary stage and are yet to be reviewed by senior management; however, using these proposed metrics, the PMT has started to analyze resource utilization trends and assess overall resource requirements, and forecast fiscal financial needs for the highly resource-intensive clinical protocol portfolio within the RCHSPP. The intent of these metrics is to provide senior management with a better understanding of the impact of resource commitments needed for various clinical, operational, and scientific initiatives.

PMT's efforts to implement a project management policy for the Ebola clinical research efforts are discussed in the "Viral and Hemorrhagic Fevers: Ebola" section of this report.

The PMT is also developing/customizing project management tools and templates (project plan, communication plan, risk register, issue register,

change control register, and project schedule) that the Ebola Project Management Team (EPMT) can leverage in managing various international projects. The PMT continues to facilitate the implementation of the project management policy that is designed to create a project management culture within Frederick National Laboratory for Cancer Research (FNLCR) that is flexible and disciplined, and is more closely aligned with industry best practices. Using FNLCR's project management guidelines, the PMT expects improved project management systems to track and monitor projects and program progress (cost, schedule, and scope), highlight issues with program execution, and facilitate the rapid resolution of those issues.

As part of the Leidos Biomed project management requirements, PMT continues to support the EPMT with preparing Interim Progress Review (IPR) presentations using the Viral Hemorrhagic Fevers: Ebola Monthly Status Report. This IPR presentation on the SharePoint site provides a snapshot of the Ebola study status in West Africa. This presentation includes a summary of the project status, changes since the last month or quarter, and a performance status overview dashboard completed independently by the program director in consultation with technical project managers.

The PMT continues to support the RCHSPP senior management, technical project managers, financial team, and functional group leaders to address emerging operational/logistical support needs and project management requirements as per the RCHSPP/OCRPRO strategic and Ebola program management initiatives.

Protocol Navigation/Protocol Development Program

Now in its seventh year, the Protocol Navigation/ Protocol Development Program (PN/PDP) has been fully integrated into the NIAID research development process. There are two aspects of this program: (1) Protocol Navigation (PN), which facilitates the research logistics that are critical to study start-up activities for protocols being conducted at the NIH Clinical Center, collaborative clinical sites, and international investigative sites; and (2) Protocol Development (PD), which involves drafting and editing protocol documents in preparation for submission to the various approving committees associated with each protocol. This involves collaboration with the OCRPRO and RCHSPP functional groups, as well as with other entities within NIAID and NIH.

PN/PDP represents an exceptional administrative initiative that has served as a model to other NIH institutes/centers, and has improved NIH program operations and benefited the NIH research environment. PN/PDP provides investigators with comprehensive protocol development support services to meet the increasing demands on clinical research related to the regulatory compliance process and efficient navigation through the myriad of clinical processes, from study concept to publication.

PN/PDP provides early interventions in protocol development through navigation processes, administrative and regulatory requirements management, and technical writing support. The protocol navigators guide PIs through the regulatory and administrative requirements to facilitate the submission process, aiming to avoid unnecessary delays. The medical writers assist PIs in originating and editing clinical protocols in any stage of document development, including study concepts, informed consent documents, amendments, SOPs, and publications. During protocol development, the team collaborates with PIs to discuss protocol implications related to regulatory requirements, NIH policies, and project timelines, as well as to enhance the overall accuracy and quality of content. Logistics management includes support to NIAID intramural investigators and research study teams with developing, writing, and tracking clinical protocols through the initial protocol life cycle (concept stage through protocol development, review, approval, and initiation). PN/PDP involvement is very helpful in keeping investigators and collaborators engaged in the often lengthy study start-up process. Investigators continue to express their support for the services that the PN/PDP team provides, citing the team's effectiveness in keeping them on track with the protocol logistics and ensuring that protocols include consistent and applicable language for IRB submission.

Protocols vary in phase, type, and sponsorship, and have also spanned most intramural labs, including the Laboratory of Clinical Infectious Diseases; the Laboratory of Immunoregulation; the Laboratory of Parasitic Diseases; the Laboratory of Allergic Diseases; the Laboratory of Immunogenetics; the Laboratory of Infectious Diseases; the Laboratory of Molecular Immunology; the Laboratory of Host Defenses (LHD); the Laboratory of Malaria Immunology and Vaccinology; Laboratory of Immunology; the Laboratory of Human Bacterial Pathogenesis; Laboratory of Systems Biology; Laboratory of Malaria and Vector Research; the Laboratory of Zoonotic Pathogens, the Laboratory of Intracellular Parasites and the Laboratory of Virology at Rocky Mountain Labs (RML); the Collaborative Clinical Research Branch; and the NIH Department of Laboratory Medicine.

Current staff members include a PN/PDP project manager, 2 protocol navigators, and 3 medical writers. PN/PDP continues to add significant value to the protocol development process by providing services related to protocol and consent drafting and logistics management, and assisting new and experienced clinical investigators.

In addition to the normal work requests that PN/PDP receives and the ongoing projects that may carry over in development from year to year, this reporting period continued to focus on the NIAID DCR high-priority Ebola research initiatives, specifically, assisting in the continuation of protocol activities related to expanding the research conducted by NIAID in West Africa. At the request of the OCRPRO director and NIAID clinical director, PN/PDP was involved with amending consents and revising flip books as a supplement to the consent. Other projects were reprioritized and reassigned to enable the team to meet the study timelines and get the requests completed in an expeditious manner for the NIAID clinical director. The PN/PDP continues to facilitate the protocol development and logistical management of an early phase rabies Ebola vaccine protocol to be conducted domestically at NIH.

During the reporting period, the PN/PDP team was involved with the development of initial review protocols and new protocol requests; of the new requests, eight were first-time users of PN/PDP services.

The working titles of the new protocol requests are as follows:

1. Incidence and consequences of herpesvirus reactivation in HIV-infected women initiating antiretroviral therapy (ART)
2. Prevalence of Clonal Mast Cell Disease in Allergen-Specific Anaphylaxis
3. A Phase 2 Pilot Study to Evaluate the Efficacy of Actemra (Tocilizumab) for Treatment of Indolent Systemic Mastocytosis
4. Symptom Grading Tool for Adults with RSV
5. LHD Stored Samples/Data
6. Preventive Immunomodulation for Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy
7. Partnership for Research on Ebola Vaccinations (PREVAC)
8. PREVAIL IV: Double-Blind, Randomized, Two-Phase, Placebo-Controlled, Phase II Trial of GS 5734 to Assess the Antiviral Activity, Longer-Term Clearance of Ebola Virus, and Safety in Male Ebola Survivors with Evidence of Ebola Virus Persistence in Semen
9. A Phase 2a Study to Evaluate the Safety, Tolerability, and Immunogenicity of One Dose of Ndv-3A Vaccine in Patients with STAT3-Mutated Hyper-IgE Syndrome
10. Studies of the Natural History, Pathogenesis, and Outcome of Autoinflammatory Diseases (NOMID/CAPS, DIRA, CANDLE, SAVI, NLRC4-MAS, Still's-like Diseases, and other Undifferentiated Autoinflammatory Diseases)
11. Compassionate Use Treatment Protocol I4V-MC-JAGA(q): Treatment of Conditions Expected to Benefit from JAK 1/2 Inhibition: CANDLE, CANDLE-Related Conditions, and SAVI
12. Microbial, Immune, and Metabolic Perturbations by Antibiotics (MIME study)
13. An Open-Label Extension Study to Evaluate The Long-Term Safety, Tolerability, Efficacy, and Pharmacokinetics of CDZ173 in Patients with APDS/PASLI (Activated Phosphoinositide 3-kinase Delta Syndrome/p110δ-Activating Mutation Causing Senescent T Cells, Lymphadenopathy, And Immunodeficiency)
14. An Open-Label, Phase I/II Pilot Study to Assess the Safety and Tolerability of Vorinostat for the Treatment of Moderate-to-Severe Crohn's Disease
15. Randomized, Double-Blind, Placebo-Controlled, Phase 1b Study in Healthy Volunteers to Evaluate the Safety and Efficacy of AGS-v, a Universal Mosquito-Borne Disease Vaccine
16. CRISPR-Gene Mutation Repair to treat Chronic Granulomatous Disease
17. Impact of Once-Weekly Rifapentine and Isoniazid on the Steady State Pharmacokinetics of Dolutegravir and Darunavir Boosted with Cobicistat in Healthy Volunteers
18. Use of Ustekinumab (Anti-IL-12/23p40 monoclonal antibody) in Patients with Leukocytes Adhesion Deficiency 1 (LAD1) Who Have Inflammatory Pathology
19. Glutamine for Caspase Recruitment Domains (CARD)
20. Chronic Granulomatous Disease (CGD) Colitis

PN/PDP also assisted with amendments, several of which were related to Ebola research; this entailed coordination of documents and multiple communications with domestic and international collaborators in order to process the approvals. Several amendments required less than a day turnaround time due to the urgent clinical need to address the cluster Ebola outbreak in Liberia. Other assignments included creating a flipbook for information regarding blood draws for people in Liberia, handling the logistics for obtaining Office of Human Subjects Research Protections (OHSRP) Program determinations regarding IRB review, and providing advice to investigators navigating the logistics of submissions.

The PN/PDP continues to be able to shift workloads and prioritize the Ebola assignments to meet the urgent demands, while maintaining customer support of the other PN/PDP research projects. The Leidos Biomed Scientific Publications, Graphics & Media department continues to assist and provide excellent support and quick turnaround deliverables with the flipbook illustrations.

The PN/PDP convenes meetings with the NIAID clinical director, the OCRPRO branch chief, and various oversight managers to keep each party up-to-date on the workload and upcoming projects, to troubleshoot issues, and to promote the future growth of this program. A monthly status call is held between OCRPRO and RCHSPP staff members to keep all aware of timelines, areas of concern, and action items. This call assists the teams with planning and evaluating future workloads.

The PN/PDP staff work closely with biotech and pharmaceutical managers in collaborating with the NIAID investigators and help these managers become familiar with the NIH policies and processes. The staff work closely with the NIAID scientific office in development of novel agents for first-in-human trials in collaboration with the Walter Reed Army Institute of Research (WRAIR) and Rocky Mountain laboratories. The Ebola projects require comprehensive and organized collaboration with Liberian representatives, the French National Institute of Health and Medical Research (INSERM) team, the University of Minnesota colleagues, and several ancillary staff involved in the ongoing and new research efforts necessary for studies such as PREVAIL IV and PREVAC.

The poster titled "Development and Implementation of a Graphic Aid to Consent for an Ebola Vaccine Trial in Liberia" was presented at the Public Responsibility in Medicine and Research (PRIM&R) Advancing Ethical Research Conference in November and was designated as outstanding by the planning committee, leading to an invitation to participate in an Innovations in Global Research Panel, which highlighted exceptional posters and provided information sharing among many domestic and international institutions.

The PN/PDP team members have been asked to provide overviews of the program and the services provided to Liberian site managers. It has been requested that PN/PDP review protocols, provide advice, and meet with Indonesian colleagues from the INA-RESPOND network.

For the fifth year in a row, the PN/PDP team was invited to participate at the Ph.D. student summer course in clinical and translational research at the NIH Clinical Center. The course involved didactic interactive sessions given by the medical writers and protocol navigators and was an introductory program for Ph.D. students (selected by NIH) with no prior experience in clinical research or human subject protocols. This session provided an opportunity for PN/PDP to demonstrate resources available within NIAID to future PIs.

Since its inception, PN/PDP has continued to receive universally positive feedback regarding its value in reducing regulatory and administrative burdens; optimizing the use of existing clinical tools within NIAID and NIH; maintaining knowledge and implementing best practices of protocol management; assessing the impact of the various steps of protocol development on clinical trial efficiency; developing metrics to identify, measure, and target processes that create opportunities and efficiencies throughout the clinical research review and approval process; and, most importantly, reducing investigator burden while facilitating communications between the multiple organizations involved in the review of clinical research. The program has been enthusiastically received by investigators, as demonstrated by the increased utilization of services, referrals, and repeat customers.

Regulatory Affairs

The RA group prepares, submits, and maintains IND applications, IDEs, and DMFs to ensure that these documents comply with federal regulations and the ICH/GCP guidelines. The RA group consists of 1 regulatory affairs director, 1 IND manager, 6 regulatory associates, and 1 regulatory submissions coordinator.

In collaboration with the OCRPRO IND clinical research oversight manager, the RA group fulfills IND, IDE, and DMF sponsorship responsibilities. Staff members provide overall regulatory support and guidance to the intramural investigators, serve as liaisons with various FDA divisions, and interact with various industry collaborators and other outside subcontractors to obtain information required to support OCRPRO-sponsored projects. The RA group supports investigators in the NIAID Intramural Research Program, which includes multiple laboratories within the Division of Intramural Research (DIR), investigators within DCR, and external investigators under contract to NIH.

Additional responsibilities of the RA group include preparing, compiling, and submitting various documents (e.g., protocol amendments, information amendments, annual reports, safety reports, and responses to FDA comments/requests for additional information) to maintain and ensure regulatory compliance of OCRPRO-sponsored INDs, IDEs, and DMFs. Staff also ensures compliance with the mandated reporting requirements for the https://clinicaltrials.gov website.

During FY2016, the RA group supported 83 IND applications, three Clinical Trial Applications (CTAs), three IDEs, five DMFs, and three single-patient emergency and non-emergency INDs (eINDs), several of which include protocols conducted at multiple sites and international locations. The group prepared and submitted 16 new IND applications; at the time of this report preparation, approximately 10 INDs/IDEs were in various stages of development. As part of the ongoing maintenance for these new and existing applications, staff developed and submitted approximately 400 IND, IDE, and DMF serial

submissions, and three pre-IND meeting requests and information packages to the FDA. Two pre-IND teleconferences were held with the FDA, one of which focused on an upcoming mosquito saliva vaccine study for the prevention of Zika virus infection. Staff also participated in many teleconferences with the FDA to discuss other IND-related issues.

Other support provided by the RA group during FY2016 included: (1) participating in meetings with NIAID scientific investigators, FDA representatives, PIs, collaborating industry representatives, and other stakeholders to discuss ongoing scientific issues and IND management strategies (e.g., bovine IgG MERS vaccine, RSV vaccine, and Ebola antiviral and vaccine studies); and (2) providing Current Good Manufacturing Practices (cGMP) guidance to OCRPRO and investigators regarding product storage, shipment, labeling, and manufacturing issues.

The RA group continued to evaluate and improve the eCTD program with the refinement of work guidelines and process modifications where appropriate. Staff expanded the eCTD product dictionary and eCTD process overview. Staff also revamped and modified the RA group Manual of Procedures (MOP) to streamline its usability and the work processes described therein.

Most members of the RA group provided support to DCR's Ebola efforts this fiscal year. Staff maintained four existing INDs and prepared and submitted three new INDs for Ebola vaccines and drug products, and dedicated significant time and resources on a pre-IND meeting and application for an Ebola-rabies vaccine. In addition, staff continued to manage the Liberian Medicines and Health Products Regulatory Authority (LMHRA) CTAs for two of these Ebola products and also compiled a new application for an Ebola antiviral drug that was delivered electronically to the PREVAIL Operations team that will be sponsoring the application in Liberia. Much of this work had to be done on very short notice and took precedence over all other projects.

The first IND Annual Reports for the PREVAIL I vaccine study and the PREVAIL II ZMapp medical countermeasures study were drafted and submitted to the FDA, and a final clinical study report (CSR) was prepared for the ZMapp study. Preparation of the CSR involved working with the study teams and stakeholders (e.g., University of Minnesota and Mapp Biopharmaceutical, Inc.) to obtain study enrollment, safety, and efficacy data.

Following the study closure in January 2016, regulatory staff began preparation of a CSR for the Ebola medical counter measures (PREVAIL II, ZMapp) study. The regulatory associate quickly prepared a 90-plus page draft report, which included specific, detailed protocol information about the study conduct and results. This was a relatively new effort for the RA group and involved research into the requirements for content and format, as well as a thorough review and understanding of the clinical protocol documents and available study data. Staff completed many of the report sections, such as the study synopsis, introduction, trial objectives, and study design, and returned a robust draft CSR to the study team for review and input in less than three weeks. The RA group submitted the final report to the FDA and Liberia and Sierra Leone regulatory authorities in July 2016.

In May 2016, the RA director and a regulatory associate traveled to Liberia to provide hands-on training to PREVAIL operations staff on the requirements for and preparation of CTAs. The regulatory team presented overviews of investigational trials and the principles behind the ethical conduct of clinical research, and reviewed the LMHRA draft guidelines for CTAs and for the Liberia pharmacovigilence system. They covered the specific contents of a CTA and the processes for application preparation and submission, as well as suggestions for archiving an application and any associated documents. Finally, an overview of the FDA, the U.S. regulatory authority governing investigational new drugs, and the regulations behind, content of, and processes regarding the development and maintenance of an IND application was provided for reference material. Complete training binders containing all of the material covered, along with templates for the various CTA components, were given to the PREVAIL team. In addition, prior to their arrival, the RA team shipped a package of CTA supplies to Liberia that included boxes of labels, tabs, blank CDs, and hard cover report folders. Having these materials on-hand allowed the team to offer step-by-step instructions for even the finest details of CTA preparation. Ultimately, four PREVAIL operations staff participated in the training and were very grateful for the information and time given them.

The RA group continued to provide regulatory support for ongoing and new influenza studies under the IRC, La Red, and INSIGHT networks, for which staff submitted approximately 20 amendments, including protocol amendments, new protocols, and drug manufacturing updates. In addition, staff participated in numerous teleconferences with DCR, NIH PIs, and other IRC, INSIGHT, and La Red study stakeholders in support of these studies.

The RA group recognized a discrepancy between the 21CFR 312 requirements and the RCHSPP/OCRPRO practices concerning the submission of IND protocol amendments. Based on this information, the director recommended that changes be made to the instructions given to investigators in No Regulatory Concern (NRC) emails and, upon concurrence from OCRPRO, modified the NRC templates and corresponding sections of the DCR IND Guidelines document to bring RCHSPP/OCRPRO into alignment with the federal requirements.

Clinical Safety Office

The RCHSPP Clinical Safety Office (CSO) provides primary professional and administrative support to OCRPRO in three distinct functional areas: scientific and clinical expertise, data and safety oversight, and medical writing. The CSO also provides surveillance, monitoring, and regulatory reporting of SAEs occurring in NIAID-sponsored clinical trials, including all OCRPRO IND-sponsored trials. The CSO ensures compliance with the Code of Federal Regulations, NIH policies, and ICH/GCP guidelines for protocols, informed consent documents, and case report forms.

Following the significant staffing challenges and role changes from the prior year, FY2016 has been notable for growth and development among the CSO staff, several of whom have either transitioned to, mastered, or expanded current roles during FY2016. Staff members have adapted readily to process and procedural changes, and have learned to flex and leverage their skills in new roles to meet the NIAID's goals and objectives.

The CSO filled a longstanding medical monitor vacancy in January 2016. The new medical monitor has a pediatric infectious disease background, and a wealth of clinical and research experience, including work as an investigator and prior work for NIAID. Additionally, the CSO administrative assistant was promoted during FY2016. A clinical safety associate moved into a medical writer II role, while also providing support to the rapidly increasing volume of work of the DSMB and Safety Monitoring Committees (SMC). Under the guidance of its improvement-focused leadership, the CSO has attained much needed depth of coverage, as staff have learned to function effectively in multiple roles within the CSO.

The CMRP SMART goal of establishing direct access to safety data for the CSO medical monitors was achieved during FY2016. Medical monitors are now able to directly review safety data in the CRIMSON system, thus enhancing their ability to interpret data, identify safety signals, and provide meaningful support and guidance to NIAID investigators.

During FY2016, 45 SAEs and three SAE/unanticipated problems were processed and completed, along with 60 updates of information on processed events. One SAE, received by the CSO in April 2016, was determined to be a suspected unexpected serious adverse reaction and resulted in a 15-day IND Safety Report submitted to the FDA. No new reports of pregnancy were processed and followed.

During FY2016, the CSO medical monitors, clinical safety associates, and medical writer performed over 101 clinical research protocol reviews, comprising 17 PI reviews, 75 amendment reviews, three site-specific informed consent form reviews, and six protocol navigator reviews. The medical monitors present all comments and edits to the PI prior to submission to the NIAID IRB. For the initial pre-IRB reviews, medical monitors perform a final review of the entire protocol for subject safety concerns, data integrity, and clinical trial design. The review process may include conference calls with investigators and other stakeholders to discuss and resolve regulatory or safety concerns in order to ensure protocols are optimally prepared for submission to the IRB and FDA.

During FY2016, the medical monitors and clinical safety associates also reviewed over 43 IND annual reports, 16 investigator brochures, and five investigator brochure amendments.

The CSO provides administrative and logistical support to the NIAID intramural DSMB. A clinical safety associate (CSA) functions as the DSMB executive secretary, coordinating teleconferences and twice yearly in-person meetings. During FY2016, the volume of DSMB-related work increased significantly. More than 63 teleconferences were conducted. At least one review was conducted to address a specific safety concern over the past year. Of note, an increased level of study complexity and an increase in internationally conducted studies during the past year have significantly affected the DSMB's work.

In an effort to improve efficiency and customer satisfaction, the CSO modified certain DSMB work practices during FY2016, including the consolidation of protocol review dates into a twice-monthly standing schedule. An additional (seventh) core DSMB member had been recruited in late FY2015, and with that addition, the DSMB has been able to consistently achieve a quorum for its reviews during FY2016. The format of the written DSMB summary was modified in order to streamline the summary preparation process and support the CSO's goal of routinely meeting pre-determined deadlines. Also during FY2016, the DSMB executive secretary began to fully utilize the TrackWise system as an organizational tool.

In January 2016, the core members of the DSMB were surveyed in an effort to evaluate program services and identify potential opportunities for growth and improvement. The clinical research oversight manager (CROM) reviewed the survey results with the board during the June face-to-face meeting. Logistical issues, particularly problems with voice quality on international calls, were identified as an area for improvement. The CSO has addressed this and identified several alternative and complementary strategies to fix the issue, resulting in a much-improved call experience during the summer 2016 face-to-face meeting.

Each year, the DSMB executive secretary plans and facilitates two day-long face-to-face meetings. During these meetings, the DSMB meets with PIs, study teams, and other stakeholders to review

protocols for safety and efficacy, and to make recommendations concerning the conduct of these studies. Meeting preparation includes distribution of highly sensitive unblinded study data. The executive secretary also creates the meeting agenda and schedule, tracks attendance, arranges for international participants to teleconference into the meeting, and ensures the NIAID facility has an accurate list of attendees with affiliations and credentials. During the meeting, the executive secretary serves as coordinator, ensuring reviews are conducted appropriately and that the meeting remains on schedule. Following the meeting, the executive secretary prepares and distributes a detailed written summary of each protocol reviewed. During FY2016, two face-to-face meetings were conducted, at which time 20 protocols were reviewed.

The CSO is also responsible for oversight, support, and facilitating teleconferences for protocols with either Safety Monitoring Committee (SMC) or Independent Safety Monitor (ISM) oversight. A CSO staff member serves as the SMC executive secretary, and is responsible for scheduling and facilitating teleconferences and for preparing and distributing a written summary of each meeting. During FY2016, nine SMC and six ISM review meetings were conducted.

The CSO collaborates with the RA and CTM groups, providing guidance and expertise to staff as requested. Additionally, CSA staff reviews all Monitoring Visit Reports (MVRs) and collaborates with the clinical research associates and medical monitors to resolve any identified safety discrepancies. During FY2016, 84 MVRs were reviewed by the CSO.

A medical writer has been a longstanding member of the CSO team. The medical writer reviews and edits consent and protocol documents as assigned. The medical writer may also play a role in reviewing and editing a range of internal and external reports and documents, including CSA SOPs and the CSO Procedure Manual. During FY2016, the medical writer also supported the DSMB and SMC in a variety of coordination and writing assignments.

The Safety Review and Communications Plan (SRCP) is an internal communications document between the PI and the IND sponsor that delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The sponsor may determine that the PI could have responsibility for periodic safety assessments during the course of a clinical trial, in which case a protocol-specific Transfer of Regulatory Obligations (TORO) is drafted, verified, and then signed into effect by the PI and the OCRPRO director. At predetermined intervals during the study's life cycle, the PI is responsible for submitting documentation of his or her review to the CSO.

As noted in a previous annual report, the CSO implemented a revised Safety Expedited Reporting Form (SERF) during FY2014. While the revised form has resulted in improved functionality and efficiency in the SAE submission process, opportunities for further improvement were identified during FY2016. An ad hoc group within the CSO is currently analyzing the SERF and recommending revisions. Potential objectives of a revised SERF would be to improve clarity and consistency in the reporting of adverse event terms and start dates, which are vital in the reconciliation of adverse events for annual reporting to the FDA.

Since FY2014, the CSO has been responsible for SAE reconciliation in the preparation of annual protocol reports submitted to the FDA. During FY2016, the CSO reviewed 43 annual reports. The CSO analyzes each report and works to resolve any identified discrepancies. It is anticipated that the planned revisions and modifications to the SERF will help expedite this process and will ensure overall accuracy in all reports.

In recent years, the CSO detected a trend toward increasing pregnancy rates among subjects enrolled in NIAID studies. In an effort to address this issue and to inform and support NIAID investigators concerning pregnancy prevention efforts, the CSO launched a Pregnancy Prevention Initiative beginning in FY2015. The initiative continued during FY2016, as pregnancy prevention templates for insertion into protocols and consent documents went through a process of peer evaluation resulting in significant revisions. Overall, the initiative, which is still underway, has thus far been well-received by NIAID investigators, and the CSO views the Pregnancy Prevention Initiative as an area of ongoing vital importance. The CROM and members of the CSO group presented a comprehensive review and didactic presentation on the Pregnancy Prevention Initiative during the 2016 Association of Clinical Research Professionals annual conference and also made a similar presentation to CMRP staff.

The CSO operations manager worked closely with the CROM to develop a Manual of Procedures (MOP) Review Guidance Checklist of potential safety-related topics in order to standardize the MOP development process. The draft was finalized during FY2016 and posted to the NIAID OCRPRO portal.

The CSO director continues to support the NIH summer Ph.D. student program, serving on the student selection committee and presenting on the topics of informed consent and protocol development. CSO medical monitors have made several presentations in FY2016, including scientific presentations on drug-induced liver injury and mosquito-borne diseases.

Division of Intramural Research

Support Provided by the Clinical Monitoring Research Program

India/Mali Initiative International Centers for Excellence Research

The India/Mali International Centers for Excellence in Research (ICER) initiative is an ongoing project sponsored by the National Institute of Allergy and Infectious Diseases' (NIAID) Division of Intramural Research (DIR) to establish an infrastructure that facilitates research relevant to the pathogenesis and control of lymphatic filariasis in both Indian and West African populations. Because India and Africa disproportionately bear the burden of lymphatic filariasis, the infections must be studied in these international locations. With few resources, the countries require outside assistance to develop sustainable research capabilities and program strategies relevant to their local conditions.

Since 2004, CMRP staff has assisted NIAID researchers with establishing research infrastructure and training investigators for both the Indian and Malian lymphatic filariasis research initiatives, and has conducted well-defined pilot projects. NIAID facilitates multiple clinical trials conducted by both intramural and extramural investigators.

The India/Mali/Cameroon effort provides dedicated off-site personnel in Frederick, MD and through an on-site research subcontract in India. The personnel coordinate activities for state-of-the-art laboratories, manage administrative concerns, track and monitor dedicated budgets, assist with logistics, provide project procurement support, coordinate administrative program-level functions, and scientifically manage research initiatives.

CMRP's overall goal for ICER is to facilitate communication and continuity for the clinical researchers in India and Mali. During the reporting period, CMRP provided logistical and administrative support for daily international operations; prepared and monitored budgets; prepared travel arrangements for nonemployees; procured miscellaneous biological and laboratory supplies; continued support of service maintenance agreements for equipment located in India and Mali; and coordinated and tracked perishable, ambient temperature, and frozen shipments to Cameroon, Chennai, and Bethesda.

The research subcontract provides a scientific director, located in Chennai, India, who oversees the research projects conducted in the United States at NIH and in India at the National Institute for Research in Tuberculosis (NIRT), International Center for Excellence in Research, formerly known as the Tuberculosis Research Center.

During FY2016, the collaborative program accomplished the following activities:

- Identified the immune changes following treatment of helminth infections
- Elucidated the role of IL-10 family of cytokines in filarial infections
- Elucidated the effect of diabetes mellitus on CD4+ and CD8+ T-cell responses in latent tuberculosis
- Elucidated the effect of pre-diabetes on T-cell and cytokine responses in both latent and active tuberculosis
- Identified circulating angiogenic factors—VEGF-A and C as biomarkers of disease severity and bacterial burden in pulmonary tuberculosis

In FY2016, CMRP staff investigated the possibility of reducing costs for the subcontract by engaging a different source for that effort. Staff obtained a cost proposal from an alternate vendor, but the proposal was significantly higher in cost than the current vendor. CMRP staff also investigated the possibility of subcontracting directly with the NIRT scientific director. Results of that inquiry are pending.

Laboratory of Immunoregulation

Support Provided by the Clinical Monitoring Research Program

Rakai Project

The mission of the International HIV and STD section (IHSS) of the Laboratory of Immunoregulation (LIR), which is part of the National Institute of Allergy and Infectious Diseases (NIAID) Division of Intramural Research (DIR), is to: (1) investigate the human immunodeficiency virus (HIV) transmission kinetics, viral evolution, natural history, and pathogenesis of HIV in developing countries; (2) define the clinical features of HIV and its co-infections, and respond with antiretroviral therapy in developing countries; (3) measure the impact of HIV/STD intervention trials on population-level incidence and public health; (4) design and test new diagnostic assays for use in the developing world; and (5) perform translational research on laboratory-derived hypotheses in collaboration with other researchers from the LIR. IHSS, LIR, has an ongoing initiative with the Rakai Health Sciences Program (RHSP) to establish the provision of antiretroviral drugs to rural villages in the Rakai District, Uganda, Africa. Since 2004, CMRP has supported RHSP by providing timely assistance with research subcontracting, purchasing, and shipping instrumentation and supplies. The Rakai Project is a NIAID International Center for Excellence in Research (ICER) initiative. An ICER is a laboratory-oriented

grant that funds many of the laboratory studies conducted on biospecimens. The primary purpose of the ICER initiative has been to build infrastructure in Rakai, Uganda for the collaborative conduct of biomedical research with Ugandan scientists. IHSS, LIR conducts this research in collaboration with Johns Hopkins University located in Baltimore, MD.

CMRP provides dedicated personnel to IHSS, LIR and the Rakai Project, both on-site in Africa and off-site in Frederick, MD; supports data analysis and manuscript writing for clinical research; provides operational support to clinical research protocol development and implementation; manages administrative concerns; provides technical and budgetary oversight of subcontracts; tracks and monitors the program budget; assists with personnel logistics; and provides project procurement support, travel support, and overall coordination of administrative program-level functions. CMRP is poised to support all international efforts in support of IHSS, LIR.

CMRP continues to provide subcontract management and administration support to the Task Order 4 agreement established with the RHSP to conduct protocol 14-I-N123, Quantitative Measurement and Correlates of the Latent HIV Reservoir in Virally Suppressed Ugandans. Initially, it was established as a cross-sectional, descriptive blood-draw study to measure the size of the latent HIV reservoir in virally suppressed, HIV-infected individuals residing in Uganda, and to examine the immunological and virological correlates of the latent reservoir. The principal investigator's (PI's) aim was to compare data on the HIV latent reservoir in this Ugandan population to data collected from U.S. cohorts to better understand the potential for an HIV cure in the African setting.

Study recruitment began during FY2014 and continued throughout FY2015 into FY2016. The initial study included the recruitment of 70 HIV-infected individuals receiving antiretroviral therapy (ART) and having suppressed viral loads of less than 40 copies per ml over a period of 10 to 18 months in Group 1, and 10 HIV-infected individuals with suppressed viral loads of less than 40 copies per ml over a period of 10 to 18 months in Group 2, for a total of 80 subjects. The study initially screened 76 participants, recruited 70 participants in Group 1, and recruited zero participants in Group 2, with no failures reported.

During FY2016, the protocol was amended to be a longitudinal descriptive study that includes follow-up visits for a three-year period and incorporates a third subject group. The amended study will analyze blood samples from 100 HIV-infected, virally suppressed individuals residing in Uganda to measure the size and immunological and virologic correlates of the latent HIV reservoir once a year (plus or minus 30 days) for three years. The slope or decay curve of the reservoir will be calculated for each individual over a period of three years of viral suppression to determine the T1/2 of the reservoir.

The amended study will examine the following three groups:

Group 1: Seventy HIV-infected individuals on ART with suppressed viral loads, defined as less than 40 copies per ml over a period of 10 to 18 months.

Group 2: Twenty HIV-infected individuals on ART with suppressed viral loads, defined as less than 40 copies per ml over a period of 10 to 18 months, who have known windows (within 18 months) of seroconversion through the Rakai Community Cohort Study survey.

Group 3: Ten HIV-infected individuals with suppressed viral loads, defined as less than 40 copies per ml over a period of 10 to 18 months, but are not on ART (elite suppressors).

CMRP successfully negotiated revised pricing with RHSP to continue to conduct Task Order 4 (protocol 14-I-N123) as amended.

During FY2016, the overall support to IHSS, LIR transitioned from one CMRP team to another. The successful transition allowed for continued support for logistical and administrative tasks related to daily international operations, budget preparation and monitoring, travel support, and procurement of equipment and miscellaneous laboratory items.

During FY2016, procurement support, including biologicals and lab supplies, was provided, which resulted in approximately 120 direct ship orders to the IHSS, LIR and the Johns Hopkins University collaborative lab located in Baltimore, MD, and approximately six international shipments. Travel support resulted in approximately 21 travel packages prepared for both international and domestic travel to project sites, conference travel, and travel for training purposes.

Rakai Biostatistician Support

A CMRP biostatistician supports the development and analysis of novel, clinical, virological, and immune assay data collected through various NIAID ICER studies conducted within the clinical laboratories and at the RHSP research station; performs data manipulation, computer programming, and statistical analysis on a broad range of clinical and laboratory studies; and creates databases to produce analysis data sets for assigned projects, including abstract, presentation, and manuscript generation.

The biostatistician maintains expertise in state-of-the-art data manipulation and statistical analyses; performs statistical analyses on data related to researching, experimenting, diagnosing, treating, preventing, and curing human diseases; consults with investigators on the design and analysis of clinical and observational studies; identifies programming

requirements and assists investigators with developing specialized programs to resolve statistical analysis problems; provides database management support; interacts with clinical and laboratory investigators in processing data, conducting statistical analyses, and writing reports for Data and Safety Monitoring Board (DSMB) and Institutional Review Board (IRB) requirements; assists NIAID PIs in protocol writing, study design, and case report form (CRF) development; actively contributes to manuscript writing; assists the NIAID PIs with protocol implementation; and ensures that the system and program documentation for assigned projects are complete and accurate.

The biostatistician's work efforts have focused on statistical and data analysis for scientific manuscripts, and data management for creating a research database to evaluate the RHSP ART program in the Rakai district, Uganda, Africa.

Scientific topics in development include the following:

- Mortality, virological, and retention outcomes of a rural antiretroviral therapy program in Rakai, Uganda, from 2004 to 2012.
- Viral load outcomes at 12 months of ART initiation are predictive of patients who are more likely to experience virological failure: case study of the Rakai ART treatment cohort (two abstracts submitted to the 21st International AIDS Conference [AIDS 2016], Durban, South Africa and the Center for AIDS Research Sub-Saharan Africa Working Group Biannual Meeting, Durban, South Africa; manuscript in progress).
- Rates of switching from first- to second-line therapy among patients undergoing ART treatment in Rakai, Uganda (two oral presentations made at the eighth University of California, San Francisco (UCSF) Collaborative East Africa Symposium, Kampala, Uganda and the 20th International Workshop on HIV, Budapest, Hungary; manuscript in progress).
- Strategies for the prevention of hepatitis B viral infection among HIV-infected adults in Uganda (three presentations made at the Conference on Retroviruses and Opportunistic Infections [CROI] 2016 in Boston, MA; the Sub-Saharan HIV-Cancer Symposium, Kampala, Uganda; and the Global Oncology workshop, California; manuscript under co-author review).
- Low rates of transmitted drug resistance among newly identified HIV-1 sero-converters in rural Rakai, Uganda (manuscript submitted to the *AIDS Res Hum Retroviruses*).

The biostatistician performed data manipulations and statistical analyses on the RHSP ART clinical cohort data sets to develop manuscripts and presentations, and developed analysis data sets for the ART treatment cohort in linkage to the Rakai Community Cohort Study dataset, to generate manuscripts and conference presentations on the following topics: descriptive analysis of the RHSP ART treatment cohort; rate of switching from first line of ART regimens to second line among the RHSP ART cohort; determinants and trends of mortality; trends and predictors of virological failure; and retention outcomes of the ART treatment cohort.

The biostatistician provided data manipulation and statistical support to the hepatitis B virus (HBV) incidence study conducted in the NIAID ICER laboratory at RHSP, in order to generate manuscripts and conference presentations. The biostatistician III also performed statistical analysis and data manipulations that have led to the development of three abstracts, two oral presentations, and one manuscript on the incidence of hepatitis B in HIV-positive patients and the prophylactic effect of hepatitis B active ART drugs on the incidence of hepatitis B in HIV-positive persons. The biostatistician also held meetings with the study PIs during the site visit in January 2016 and developed plans for further analyses from this study.

The biostatistician provided statistical and data manipulation support to the collaboration between the NIAID ICER program in Uganda and the HIV clinic at Mengo Hospital in Uganda to analyze secondary data collected in the HIV care program for use in manuscripts, abstracts, and scientific presentations.

New work efforts introduced during the reporting period included providing statistical analysis and data manipulation support to the collaboration between the NIAID ICER program in Uganda and the Department of Medicine, Mulago Hospital to perform an analysis for the effect on mortality outcomes of initiating ART treatment in HIV/tuberculosis patients within two weeks of starting tuberculosis treatment, and supporting protocol developments on the following topics: point-of-care viral load monitoring in HIV patients on ART, and the impact and mechanism of herpesvirus reactivation on mucosal and systemic inflammation in HIV-infected women initiating ART.

HCV Viral Load among PWID in India

A major component of LIR's research efforts is the study of the immunopathogenic mechanisms of HIV infections and disease progression. During the review period, new work was initiated under the new Indefinite Delivery/Indefinite Quantity (ID/IQ) contract, Population hepatitis C virus (HCV) Viral Load among People Who Inject Drugs (PWID) in India. This research supports the IHSS, LIR, NIAID DIR.

Globally, of the 135 million people chronically infected with HCV, approximately 85 percent reside in low- and middle-income countries. Over the past few years, tremendous progress has been made in the development of HCV therapy, which has sparked optimism for the potential to eradicate or eliminate HCV from some populations. However, to achieve eradication, or even global control of HCV infection, it is essential that these new agents reach all populations, including individuals residing in low- and middle-income countries.

People who inject drugs bear a disproportionate burden of HCV infection—often times, 30–40 times the burden in the general population. Furthermore, among HIV-infected drug users, the prevalence of HCV infection is routinely upwards of 70 percent, and in certain areas, it is closer to 90 percent. While there have been several ongoing efforts to improve access to antiretroviral therapy among PWID in low- and middle-income countries, ignoring HCV infection will only temporarily improve survival in this population. Over time, HIV-infected persons will develop and die from HCV.

The LIR sought CMRP's support to expand and build upon the information obtained under a previous cluster-randomized trial among PWID in India. This initial study revealed a high burden of HCV disease (37 percent) and lack of services for this population (Solomon et. al., *Lancet Infect Dis*, 2015). Stored specimens from the baseline assessment of this study were used to determine the presence of antibodies to HCV, and the HCV care continuum in this population was evaluated. Only 7 percent of HCV-positive individuals had ever been tested before, mostly because they had never heard of HCV. Among the HCV-positive individuals, only 5.5 percent were aware of their status and only 1.4 percent had taken any treatment. These preliminary findings clearly highlight the high burden of HCV infection among drug users in India with poor access to HCV diagnostics and treatment.

To support the Population HCV Viral Load among PWID in India effort, charter and project management plans were developed, and a sole-source justification, New Work Request Packet, and Statement of Work (SOW) were prepared for the acquisition of a subcontract with YRG CARE. A kick-off call for the project team was held where the charter was presented and project plans were discussed. During the project kick-off meeting, the sequential order of the objectives was rearranged. This change reflected an underlying priority aligned to the way the work will be executed. Subsequently, the NIAID project officer/project lead requested additional changes in scope, with further changes to the objectives required. The changes resulted in the second modification to the ID/IQ Task Order (TO).

The SOW was incorporated into the Request for Proposal (RFP) that was sent to YRG CARE in India. YRG CARE submitted a proposal in response to the RFP, which was evaluated and subsequently resulted in a subcontract award to YRG CARE. The subcontract included the following objectives that align to the ID/IQ SOW:

1. Measure the population level HCV viral load and determine the frequency of viral clearance among PWID in India.

   The information will then be examined to determine the association between community HCV viral load using different measures (e.g., prevalence of detectable HCV RNA in a community, average log10 HCV RNA in the community, etc.). HCV RNA viral load testing will be performed on approximately 5,000 samples.
2. Implement and evaluate the inclusion of HCV literacy and education programs and HCV antibody testing programs into the existing Integrated Care Centers (ICCs) to determine if these programs will improve knowledge of HCV and awareness of HCV status among PWID in India. The HCV literacy program and the antibody testing will be conducted at approximately six ICCs.

   Objective 2 will be broken out into the following two sub-objectives:

   i. HCV Literacy and Education Program—Evaluate the impact of HCV literacy and education programs and free HCV antibody testing on awareness of HCV status (by self-report) to compare the effectiveness of including these services in the ICCs.

   ii. HCV Antibody Testing—Serological testing will be performed on approximately 18,000 samples.

During the evaluation of the price proposal submitted by YRG CARE, a discrepancy in the company's calculations was identified and communicated to YRG CARE during the Best and Final Offer process. Also included in the Best and Final Offer communication was a request to re-evaluate and reconsider the HCV RNA testing method and associated pricing proposed. As a result of identifying the discrepancy and negotiating the HCV RNA test pricing, YRG CARE's overall price proposal was reduced and brought into alignment with the project budget.

Management of the subcontract with YRG CARE continued throughout the reporting period.

Laboratory of Malaria Immunology and Vaccinology

Support Provided by the Clinical Monitoring Research Program

In FY2013, NIAID asked CMRP to provide clinical and operational program support, including administration and management of a subcontract, to the Laboratory of Malaria Immunology and Vaccinology (LMIV) for the conduct of a longitudinal clinical study on pregnant women and malaria in Rakai, Uganda. The study is titled Collection of Biological Material from Pregnant Women in a Highly Endemic Region for Plasmodium falciparum (13-I-N074). Leidos Biomed awarded Task Order (TO) No. 02 to the Rakai Health Science Program in 2014 to conduct this study, which was initiated the same year.

Malaria during pregnancy is associated with low birth weight, maternal anemia, and gestational hypertension; both inflammation and the fetal response to infection may contribute to these poor outcomes. The study was designed to identify pregnancy malaria vaccine candidates who will elicit antibodies with functional activity similar to that of naturally acquired antibodies. Pregnant women were recruited into a cross-sectional study conducted in Rakai, Uganda. However, the LMIV principal investigator (PI) halted the study early in FY2015 due to the study objectives not being met. The goal of the study was to recruit 1,500 pregnant women in the cross-sectional study, and at the time the study was stopped, 933 subjects had been enrolled, with 929 completing the study.

CMRP was also requested to provide administration and management support to a subcontract for another LMIV longitudinal clinical study, Impact of Trimethoprim-sulfamethoxazole Prophylaxis on Malaria Infection and Immunity in Children in Uganda (14-I-N073). The study was designed to research the effects of drugs that have been shown experimentally to kill malarial parasites during the liver stages of infection and provide immunity in Ugandan children. Leidos Biomed awarded TO No. 03 to the Rakai Health Science Program to conduct the TMP-SMX study. Malaria remains one of the most significant causes of morbidity and mortality globally, and recent studies indicate that drugs used in HIV management (e.g., TMP-SMX) can have antimalarial properties.

The exploratory objectives of the study are to examine the effects of TMP-SMX prophylaxis on measures of malaria infection and immunity, comparing children who are taking the prophylaxis to those who are not. The study planned to enroll up to 70 HIV-uninfected, HIV-exposed children and up to 100 HIV-uninfected, HIV-unexposed children. The study completed full enrollment by the enrollment end date of July 31, 2015. The study screened 174 participants and recruited the 170 subjects as anticipated.

In response to LMIV's request, CMRP provided support for all clinical, operational, and administrative aspects of this study, including: (1) establishing the scope of the project(s) required for study implementation; and (2) developing an implementation plan with defined milestones, deliverables, and performance evaluation criteria.

CMRP successfully performed study close-out activities and subsequently closed out both Rakai Health Science Program TOs No. 02 (13-I-N074) and No. 03 (14-I-N073).

Laboratory of Host Defenses

Support Provided by the Clinical Monitoring Research Program

The main focus of the Laboratory of Host Defenses (LHD) is to study immune functions essential for host defense against infection, as well as the genetics and pathophysiology of inherited primary immune deficiencies. These abnormalities may be associated with recurrent infections and/or the dysfunction of immune homeostasis, which the laboratory investigates through clinical protocols. LHD clinical investigations aim to develop new diagnostic and therapeutic approaches to the management or correction of immune dysfunction.

CMRP provides research subcontract oversight and management, as well as program/project management by collaborating with the LHD project lead to develop plans and negotiate subcontracts with various vendors for master cell bank development, safety testing, and, ultimately, vector production.

It is imperative to the study of immune function and the genetics and pathophysiology of inherited primary immune deficiencies to have Good Manufacturing Practices (GMP)–grade, disease-specific vectors produced for use in clinical trials. These vectors must be validated and undergo safety testing to ensure subject safety. CMRP established several research subcontracts and purchase orders to support LHD and assist with developing collaborative relationships with various vendors to address scientific immunological questions about a wide range of diseases/dysfunctions, including X-linked severe combined immunodeficiency (XSCID) and chronic granulomatous disease (CGD).

CMRP managed and closed out one subcontract and two purchase orders established to support the production of master cell banks that will produce a lentivirus vector under GMP conditions for the clinical treatment of patients with CGD. The subcontract with Clongen was established to generate a master cell bank for two cell lines (GPRTGp47 and GPRTGgp91); however, only the GPRTGp47 master

cell bank was completed during the reporting period. The GPRTGgp91 master cell bank is anticipated to occur in the next fiscal year, and a new agreement will be established to support the GPRTGgp91 cell line. The purchase orders that were managed and closed out during the period were with the Indiana University Vector Production Facility and BioReliance. The Indiana University purchase order was established to perform biosafety testing on the GPRTGp47 cell lines produced by Clongen, and the BioReliance purchase order was established to perform in vivo and in vitro testing for virus screening of the producer cell line.

CMPR continued to manage the blanket purchase order agreement with the National Marrow Donor Program (NMDP), which was renewed for another year. A CMRP secretary III assisted the nurse managing the NMDP program with consolidating several spreadsheets to perform a cost analysis across several years of the program.

Laboratory of Clinical Infectious Diseases

Support Provided by the Clinical Monitoring Research Program

The Laboratory of Clinical Infectious Diseases (LCID) conducts clinical and basic studies of important human infectious and immunologic diseases. One such research effort is focused on adults of Asian ethnicity without HIV infection yet with autoantibodies to interferon gamma (IFNγ); all of these individuals presented with nontuberculous mycobacterial disease and other opportunistic infections. The syndrome was first recognized in 2004. An observational study was launched in 2009 to follow patients with this syndrome, investigate the origins of their autoantibodies, and examine potential immunogenetic factors influencing the development of this disease and other intracellular opportunistic infections. The results of this study could contribute further to the knowledge and understanding of the immunology of mycobacterial and other opportunistic infections in HIV-negative adult hosts.

Through a subcontract with Khon Kaen University, CMRP provided clinical and operational support for the conduct of the study, titled Mycobacterial and Opportunistic Infections in HIV-Negative Thai Patients Associated with Autoantibodies to Interferon-γ. CMRP provided programmatic support for the administration and management of the research subcontract. In addition, the clinical monitoring activities for the study were performed under a separate agreement with a clinical research organization, which is noted under the Clinical Consulting and Support section of this report. The multi-year study was conducted at three sites in Thailand: (1) Ramathibodi Hospital, Mahidol University, Bangkok; (2) Srinagarind Hospital, Khon Kaen University, Khon Kaen; and (3) Siriraj Hospital, Mahidol University, Bangkok. The study closed at Ramathibodi Hospital and Siriraj Hospital yet remains open at Srinagarind Hospital, Khon Kaen University.

CMRP modified the Khon Kaen University agreement to allow follow-up visits for enrolled study subjects through September 30, 2016. The Khon Kaen University agreement was also modified during the reporting period to expand the types of laboratory testing being performed. The Khon Kaen University agreement was extended to cover services through FY2016 and a modification to extend the agreement into the next fiscal year was issued prior to the end of the current fiscal year.

CMRP also has a task order–type agreement with Pharmaceutical Product Development (PPD) to provide clinical trial monitoring activities in support of the study. The agreement was extended to cover monitoring activities through September 26, 2016. PPD is anticipated to complete one monitoring visit during FY2016. A modification to extend the agreement into the next fiscal year was issued prior to the end of the current fiscal year.

Laboratory of Parasitic Diseases

Support Provided by the Clinical Monitoring Research Program

Repository Support

The Laboratory of Infectious Diseases (LID) has a long history of vaccine development and identification of new agents of viral diseases. LID is noted for undertaking high-risk, high-reward programs that require extraordinary time and resource commitments, such as programs to develop vaccines for viral hepatitis, severe childhood respiratory diseases, and viral gastroenteritis. Clinical studies complement LID's major areas of research, including testing candidate vaccines in clinical trials, a human challenge study with influenza to study pathogenesis and immune correlates for protection against the virus, and studies of severe virus infections in persons without known immune deficiency.

CMRP provides management oversight and programmatic support for a subcontract with Fisher BioServices to ship and store clinical research material to domestic and international locations for LID. During the reporting period, CMRP organized 40 domestic and three international shipments. This type of support involves communicating with the PI, preparing request forms, arranging shipments, tracking shipments, assuring that template data is received/reviewed, and making sure that all required documents are completed and returned. Staff also monitors the budget and provides monthly estimates-at-completion.

Vaccine Research Center

Support Provided by the Clinical Monitoring Research Program

CMRP and the Vaccine Clinical Materials Program (VCMP) have partnered to address the Vaccine Research Center's (VRC's) business needs that span the technical expertise of two Leidos Biomed directorates. By collaborating within directorates, VRC is able to take its vaccine candidates from manufacturing into clinical trials while maintaining seamless support from Leidos Biomed.

Clinical Trials Program

The Clinical Trials Program within the Vaccine Research Center requested Leidos Biomed to extend its comprehensive support to NIAID's clinical research enterprise by recruiting and hiring a multidisciplinary team of clinical professionals to provide additional medical support and project oversight to VRC Phase I and II clinical studies. These studies test, develop, and advance alternative approaches to generating protective immunity against emerging and re-emerging infectious diseases. The clinical trials, conducted at domestic and international sites, are targeting Zika, Ebola, chikungunya, dengue, and other emerging viruses.

CMRP's programmatic support services will help address global public health threats by supporting NIAID-sponsored VRC infectious disease-related clinical research protocols with the expertise to oversee the conduct of clinical trials, assist with protocol development and management, provide direct care to protocol participants, work with VRC research teams to provide pharmaceutical care, and monitor clinical trials safety data. To accomplish this, CMRP will recruit and hire: 1 physician, 1 physician extender, 1 clinical pharmacist, 1 protocol nurse coordinator, 1 clinical research nurse, 1 protocol navigation manager, 2 protocol navigators, and 1 clinical project manager. These personnel will primarily support VRC extramural clinical research activities and provide medical support for subjects on infectious diseases-related clinical studies sponsored by NIAID, with emphasis on activities related to trials conducted or vaccine and antibody products developed by Leidos Biomed.

As this is a newly received request from the VRC and there are several positions to fill, Leidos Biomed is proactively employing creative ways to strategically recruit personnel with the appropriate clinical research expertise. Efforts currently underway include exploring opportunities to attend trade shows and specific disease-related symposiums where potential candidates are likely to be present using a newly designed ad to announce position openings on various social/business media sites, exploring job posting opportunities with relevant research organizations/member societies, and expanding outreach efforts of the Leidos Biomed Human Resources Department to incorporate recruitment channels beyond those traditionally used.

Ebola Mali I and Mali II Studies

The VRC seeks to facilitate the development of vaccines against diseases caused by pathogens that threaten public health. In 2014, VRC sought support from Leidos Biomed to evaluate Ebola vaccine candidates that are safe, immunogenic, fast-acting (for acute protection), and durable (for long-term protection). CMRP partnered with the Vaccine Clinical Materials Program (VCMP) to provide seamless support to VRC, facilitating the center's objective of taking vaccine candidates from manufacturing into Phase I clinical trials with a population cohort that has the potential to be affected by the Ebola virus.

CMRP coordinated multiple activities to initiate two vaccine trials in Mali, West Africa (CVD01—a Phase I trial of a novel monovalent Ebola Zaire candidate vaccine, cAd3-EBOZ in Malian adults, and CVD02—a Phase I trial of the novel bivalent Ebola Zaire and Ebola Sudan candidate vaccine, cAd3-EBO, in Malian adults) and provide full clinical trial support. This included collaborating with VCMP, VRC clinical study teams, and VRC collaborators, as well as working with Leidos Biomed Research Subcontracts to establish subcontracts for the provision of data management, statistical analysis, and operations support services. The CMRP team consists of a clinical project manager to provide technical oversight and ensure effective communications with the project teams, VRC leadership, VCMP and CMRP leadership, site staff, support organizations, and the two subcontractors managed by CMRP.

A total of 151 participants were enrolled in the two trials. The CVD01 and CVD02 protocols were amended in December 2015 to include follow-up visits at 9 and 12 months post prime booster/placebo administration. Associated subcontracts were modified to reflect the extended period of performance for participant visits, database lock and analysis activities. Funding for two of the subcontracts transitioned from the OTS contract to an Indefinite Delivery/Indefinite Quantity (IDIQ) contract without interruption to study activities.

CMRP and VCMP have provided coordinating resources to support data and sample collection for analysis by VRC-designated laboratories and experts. Additionally, CMRP has provided an effective mechanism to keep all stakeholders informed with regular updates about trial status, study activities, and subcontractor performance related to clinical trials

monitoring, data management, statistical analysis support, and development/delivery of clinical study reports. All CVD01 immunology samples have been sent to the NIAID Vaccine Immune T-Cell and Antibody Laboratory, and the CVD01 database lock occurred in August 2016. CVD02 is expected to close in early FY2017.

Chikungunya Vaccine Trial

In FY2015, the NIAID Vaccine Research Center (VRC) requested the coordinated services of CMRP and the Leidos Biomed Vaccine Clinical Materials Program (VCMP) to support the clinical evaluation of VRC's chikungunya vaccine candidate, VRC-CHKVLP059-00-VP. CMRP rapidly conducted study initiation activities to facilitate the conduct of a Phase II clinical study in the Caribbean that aims to enroll 400 subjects. The details of these activities were described in last year's Annual Report.

Six sites, including two located in French territories (Martinique and Guadeloupe) where VRC wanted to establish relationships, were identified for participation. CMRP completed site initiation visits, activated six sites to screen and enroll subjects, and conducted 11 site visits. CMRP successfully facilitated obtaining the required French regulatory approvals for the sites in French territories. These sites received approval by ANSM (France's National Agency for the Safety of Medicines and Health Products) and the Committee for Personal Protection to participate in this study, an accomplishment that involved significant involvement by VRC, CMRP, VCMP, the sites, and the Leidos Biomed subcontractors. Activation of the Martinique and Guadeloupe sites is pending final Institutional Review Board (IRB) approvals.

As of August 2016, the study had enrolled 270 subjects. All 400 subjects were expected to be enrolled by September 30, 2016. Three of the four activated sites will enroll more subjects than originally planned if the sites in the French territories are unable to be activated in time to meet the trial timelines.

CMRP continues to manage two subcontracts related to the ongoing study activities and works closely with VRC to define processes for this multi-center international study. A study deviation issue at one of the sites was quickly addressed, with CMRP helping to define solutions and implement new processes at the site. The team also developed a study product transfer process suitable for the FDA audit of an Investigational New Drug (IND) product when the study agent at one site needed to be transferred to a different site. The process development included coordination with VCMP, the sites, and our subcontractors.

In addition to the activities identified at the start of the project, CMRP is working with a subcontractor to create and maintain the VRC 704 Trial Master File.

CMRP and VCMP hold regular meetings to coordinate VRC projects spanning both groups. Processes have been aligned to provide VRC information in a streamlined manner. CMRP maintains a Lessons Learned document to house valuable information that can be used for future projects by VRC and/or Leidos Biomed; for example, processes defined for the CHIK study will be translated into future VRC studies involving other vaccine candidates.

Zika Vaccine Trial

To support NIH's response to the Zika flavivirus, an emerging public health threat of international concern, NIAID is actively pursuing multiple ways to prevent Zika infection. In February 2016, NIAID directed VRC to immediately develop Zika vaccine candidates for testing in advanced clinical trials to speed the development of safe and effective vaccines. This effort could help control current and prevent future Zika virus outbreaks. VRC requested CMRP begin planning for a Zika vaccine trial to begin in FY2017. CMRP is well-positioned to respond to this request by leveraging existing resources and infrastructures that are used to manage other VRC research projects.

VRC plans to conduct a multi-center Phase IIB, randomized, placebo-controlled, DNA-based Zika vaccine trial at sites located in endemic or potentially endemic areas, enrolling 2,400 Zika-negative subjects. Leidos Biomed expects full funding to support the initiative by late FY2016. CMRP's experience rapidly starting study activities for VRC's CHIKV trial has optimized initial planning efforts and will ensure CMRP's ability to efficiently initiate the Zika trial at both domestic and international sites.

The study will use a two-injection regimen to evaluate vaccine efficacy in healthy adults (18–35 years of age) and magnitude/frequency of ZIKV-specific antibody response; a secondary objective is to compare incidence rates of Zika virus infection in vaccine and placebo recipients. CMRP is positioned to successfully support VRC's research efforts by providing the following services: site identification and evaluation, study-wide programmatic and administrative support, data management, statistical analysis, regulatory support, clinical monitoring, site contracts, and clinical site and laboratory support.

To date, CMRP has contacted potential sites to discuss capabilities for meeting the study's requirements and accelerated timelines, considered potential subcontractors for procuring study support services, examined trial management needs, participated in frequent planning discussions with VRC, and collaborated with Leidos Biomed VCMP and Research Subcontracts to ensure a rapid response to study initiation.

CMRP will ensure that VRC has the best opportunity to work with experienced collaborators and meet the robust study timeline. Key to this process is the efficient site identification strategy, which will utilize existing relationships with sites in other NIH clinical trial networks, focus on sites that can use a central IRB, and include sites in the Caribbean that have successfully implemented the CHIKV trials currently managed by CMRP. Additionally, subcontracts will be quickly established to immediately provide site evaluation services and a limited competition will be used to secure the other necessary subcontractor support services.

Support Provided by the Vaccine Clinical Materials Program

The Vaccine Clinical Materials Program (VCMP) manufactured multiple products to good manufacturing practice (GMP) standards, including hemagglutinin ferritin (HA-F) flu nanoparticles, HIV broadly neutralizing monoclonal antibody (bnMAb), plasmid DNA (pDNA), and numerous cell banking activities. Three mammalian master cell banks (MCBs)—two anti-HIV and one anti-RSV—as well as two associated end-of-production cell (EPC) banks were produced. Each frozen MCB included at least 500 vials. To support Zika pDNA vaccine production, two bacterial MCBs were produced. Using one MCB bank, four lots of Zika pDNA bulk drug substance (DS) were manufactured at a 100L fermentation scale. Additional pDNA bulk production included A/Indonesia raw material component and A/Singapore flu bulk DS. The core emphasis in cell culture manufacture was anti-HIV mAb (VRC01) production. A total of six 2000L scale bulk DS lots were successfully produced, including one lot with a new process that yields twice as much per lot. Also manufactured during this period were one Chikungunya (CHIK) virus-like-particle (VLP) lot, one HA-F nanoparticle (A/New Caledonia) lot, and one anti-RSV recombinant protein lot.

Fill/finish operations manufactured six lots of vialed VRC01 anti-HIV monoclonal antibody drug product (DP) totaling over 29,000 vials thus far, with one additional fill planned. The projected total of vialed VRC01 mAb in this period will exceed 37,000 vials among two presentations (2.25 ml in 3 ml vials, and 6.25 ml in 10 ml vials). The fill/finish group also manufactured a trivalent Western, Eastern, and Venezuelan equine encephalitis (WEVEE) VLP vaccine drug product (1,214 vials), a Zika pDNA vaccine (664 vials initially, with 7,200 additional vials projected), and an anti-RSV/DS-Cav1 vaccine drug product (1,000 vials projected). Additionally, a CHIK VLP reference standard (819 vials) was filled to support Quality Control (QC), and three aseptic media qualifications were completed to ensure continuity of filling operations.

QC completed method transfer activities to support six products: HA-F A/New Caledonia, CHIK VLP, WEVEE trivalent DP, VRC01 DG44 monoclonal antibody, DS-Cav1 fusion protein, and HA-F A/Singapore DP. Method qualifications were completed to support the release of these products in addition to the qualification of a residual kanamycin assay to support pDNA. Reference standard characterization was completed for VRC01 DG44 and CHIK. Eighty-six stability studies were active for DS and DP lots for VLP, HA-F, pDNA, mAb, and cAd3 product types and buffers. Stability testing of intermediate buffer solutions was completed, which has allowed for improved flexibility in expiry dating. The total number of samples tested by QC for in-process, release-of-validation purposes is 1,961. Environmental monitoring and utility testing totaled 25,344 samples. A total of 24 new analytical methods were qualified in this period, and a total of 12 methods were either developed in-house or transferred into QC. The stability testing effort included the initiation of 15 new stability protocols and the issuance of 65 interim reports. A VCMP visual inspection project initiative, comprising in-house subject matter experts, was put in place to optimize the pilot plant's visual inspection processes/procedures for vialed DP in accordance with industry standards and regulatory requirements.

Quality Assurance (QA) Lot Release completed the review and release of 27 lots, including VRC01 MCB DS and DP lots; Zika MCB and DS and DP lots; DS-Cav-1 MCB; one HA-F A/New Caledonia DS lot; one WEVEE VLP DP lot; and one Alhydrogel adjuvant lot in addition to the AAV8-VRC07 DS and DP lots filled at a subcontractor. QA on-the-floor support was completed to aid manufacturing during operations and to perform real-time production review of the process batch records. QA compliance qualified eight vendors by on-site audits, including three critical raw material suppliers and four software vendors. The VCMP shipped a total of 10,479 vials of released drug products to clinical sites and/or distribution centers during this period. VRC01 mAb represented 88 percent of the total quantity shipped, 70 percent of which was the 2.25 ml fill and 30 percent was the 6.25 ml fill configuration. The remainder of vials shipped was for other drug products including VRC01LS mAb, CHIK VLP, ZIKA pDNA, MVA Ebola, and PBS placebo.

QA/Regulatory Affairs (RA) drafted the Chemistry Manufacturing and Controls (CMC) sections for the VRC07-523LS, AAV8-VRC07, Zika DNA, and WEVEE drug products in order to successfully meet the client's timeline for submission to the U.S. Food and Drug Administration (FDA). RA also drafted responses to FDA's CMC comments for VRC01LS, VRC07-523LS, and AAV8-VRC07, in addition to France's National Agency for the Safety of Medicines and Health Products (ANSM)

CMC questions for CHIK VLP to enable progression of planned clinical trials in Guadeloupe and Martinique. QA/RA is currently drafting and compiling the CMC section for the Ds-Cav-1 vaccine and the VRC01 amendment.

During this period, QA provided a swift response to a client's request to answer the Red Team Questionnaire. VCMP received the questionnaire on the evening of Friday, April 15, and the completed questionnaire was due for submission by 9 a.m. on Monday, April 18. On Thursday, April 21, the facility provided the Red Team with a facility tour of the Pilot Plant.

Equipment validation completed the qualification or requalification of 183 unique pieces of equipment. Computer systems validation released seven systems, including the scientific alarm system, building automation system, electronic document management system, electronic learning management system, Graph Pad, regulatory asset manager, and Mobile Data Acquisition (MODA) systems upgrades.

Scientific subcontracting support was provided to supplement client research and development, manufacturing, and preclinical activities. A total of 50 new subcontracts were awarded, and in total, 87 subcontracts were managed throughout the course of the year, including subcontracts with universities, contract research organizations (CRO), contract manufacturing organizations (CMO), consultants, and other service providers. Of note, successful GMP production and release of vialed AAV8-VRC07 product was accomplished at a CMO supporting a high-visibility program at the National Institutes of Allergy and Infectious Disease (NIAID) and Vaccine Research Center (VRC). Additional subcontracting support was secured at another CMO for GMP production of anti-HIV bnMAb, 10-1074. Preclinical studies were completed and/or initiated at CROs in support of anti-HIV bnMAbs VRC01LS and VRC07-523LS, as well as WEVEE, DS-Cav1, and HA-F vaccine products. Multiple agreements were secured to support research and development for new vaccine targets and technologies, including vaccines targeting the Zika virus. Additional scientific subcontracting activities to support new indefinite delivery/indefinite quantity (IDIQ) task orders were initiated, including malaria and tuberculosis programs.

Support Provided by the Cancer Research Techology Program

Support Provided by the Electron Microscopy Laboratory

During FY2016, the Vaccine Research Center (VRC) has been supported with one full-time equivalent (FTE) employee doing negative staining analysis. A total of about 700 samples have been analyzed during this time. Electron Microscopy Laboratory (EML) efforts contributed to four patent applications. Recently, the VRC had more need for plastic-embedded samples, and the Vaccine Pilot Plant (National Institute of Environmental Health Sciences [NIAID]) started sending samples for EM analysis that are usually outsourced to other places. Due to this increase in workload, another FTE was added to the Yellow Task, and EML hired a research associate to join the team.

Over the last year, the EML has improved the image analysis capabilities for negative stain data, and in particular developed and optimized workflows for the random conical tilt method, a method by which sample heterogeneity can be observed in an objective way and that can also be used to improve particle angle distribution in cases of preferred orientation on a carbon film.

NIAID Extramural

Division of Acquired Immunodeficiency Syndrome

Chinese Clinical Trials Network

Support Provided by the Clinical Monitoring Research Program

The National Institute of Allergy and Infectious Diseases (NIAID), the lead National Institutes of Health (NIH) institute for tuberculosis (TB) research, has developed collaborations with the Chinese Ministries of Health (MOH) and other funding agencies in various countries to conduct high-quality clinical research on TB. With the second-largest TB epidemic in the world and the largest number of patients with multidrug-resistant TB (MDR-TB), China is a major priority. NIAID established a partnership with the investigators currently funded by the Chinese MOH to build a sustainable Chinese research network/consortium for the conduct of multicenter clinical TB studies. It is anticipated that several benefits will be realized from this network, such as: (1) the positioning of China as a global leader in TB research, with the necessary infrastructure and capacity to conduct high-quality, collaborative clinical research; (2) the establishment of a mechanism by which pharmaceutical companies and other collaborators can more efficiently and effectively launch TB clinical trials in high-prevalence regions; and (3) the potential for the network to serve as a platform to empower and engage the Chinese investigators to develop a scientific agenda that identifies and prioritizes TB research of global health importance.

To foster this collaboration with the Chinese, an integrated approach has been applied that focuses on the following key disciplines: regulatory processes

and standards, clinical management, laboratory and specimen management, research pharmacy management, and data management. A framework for assessing capabilities, training to enhance capabilities and address capability gaps, for establishing a clinical research management infrastructure, and for periodically evaluating performance through quality assurance review encompasses all key disciplines. The first phase of the project involved a focus on capacity building and the overall development of the network/consortium infrastructure to support network-wide operations; the second phase involves conducting TB clinical research studies and more fully establishing the network/consortium to advance the network and its sites to a level of operational independence.

The Division of Acquired Immunodeficiency Syndrome (DAIDS) leverages Leidos Biomed/CMRP as an experienced program in establishing international network, to assist the DAIDS staff in this collaboration with Chinese investigators to build a clinical trials network encompassing multiple sites in China that will conduct studies on MDR-TB.

During FY2016, CMRP supported the creation of the Chinese Tuberculosis Clinical Trials Consortium (CTCTC) initiative by providing technical expertise and programmatic oversight. Two clinical project managers oversaw all aspects of program planning and performance; these efforts included project management and reporting, procurement and budget oversight, communications, travel, and logistical support. In this role, CMRP provided oversight and functioned as an advisor to the subcontractor (FHI 360) to ensure activities for this effort were in line with NIAID DAIDS' expectations. CMRP shared knowledge and expertise with the CTCTC project team to help build capacity and expand the Network.

During FY2016, CMRP oversaw activities conducted by Family Health International (FHI) 360 in support of the following clinical trials:

- 4.5-month Regimen for Treating New Sensitive Pulmonary TB: A Randomised Controlled Non-Inferiority Trial (MOST study) – The objective of the MOST study is to evaluate the safety and efficacy of a 4.5-month regimen compared to a standard six-month regimen for the treatment of sensitive pulmonary TB.
- Clofazimine study (currently on hold)**:** The Clofazimine study is a randomized controlled trial to evaluate efficacy and safety of a nine-month clofazimine containing regimen in comparison to a standard 18-month regimen for the treatment of multi-drug–resistant tuberculosis.

In April 2016, the research subcontract with FHI 360 was modified to expand the scope of work supporting the CTCTC, increase the total budget ceiling amount of the research subcontract and revise the price schedule, and extend the research subcontract period of performance to September 2016. In July, CMRP worked with the Leidos Biomedical Subcontracts Department to modify the research subcontract to extend through FY2017.

MOST Study

FHI 360 provided scientific, quality management (quality assurance and control), and clinical monitoring support.

In January 2016, FHI 360 facilitated a teleconference with NIAID DAIDS, CTCTC, and other scientific experts to discuss the drug regimens and design for this study. Prior to an investigators' meeting in March 2016, FHI 360 worked on checklists (laboratory, data, and clinic) associated to the Minimum Standards for CTCTC Network TB Clinical Trials. These standards were created to help guide CTCTC in setting quality standards for their clinical studies. These checklists were reviewed and input was provided by NIAID DAIDS and CMRP. Based on these standards, FHI 360 introduced a clinical quality management plan to investigators and site staff who attended the Investigators Meeting.

Approximately 3,900 subjects are planned for enrollment, which will begin in September 2016, through 36 participating sites in China. Five of the 36 sites are CTCTC Network sites supported by Leidos Biomed: (1) Beijing Chest Hospital; (2) Shanghai Pulmonary Hospital; (3) Wuhan Institute for TB Control (Wuhan Pulmonary Hospital); (4) Shenyang Chest Hospital; and (5) Tianjin Haihe Hospital.

Clofazimine Study

During FY2016, the Clofazimine study was put on hold while the CTCTC Network prepared to conduct the Standardized Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR-TB (STREAM) study. The STREAM study is not supported by Leidos Biomed.

During FY2016, CMRP provided oversight for the following activities that were focused on future expansion of the Network:

- In December 2015, the FHI 360 team traveled to Cape Town, South Africa and attended the 46th Union World Conference on Lung Health. The team hosted a CTCTC networking meeting to provide a status update to stakeholders regarding the Network's progress.
- FHI 360 had a meeting with Dr. Zhi Hong, head of GlaxoSmithKline's (GSK) Infectious Diseases Research and Development Division in March 2016. The meeting was arranged as a continuation of the earlier interaction with GSK. Dr. Hong is currently interested in collaborating with CTCTC and using the CTCTC as a potential platform for GSK future studies.

- The FHI 360 team also met with Janssen Pharmaceuticals in Shanghai, China. The purpose of the meeting was to discuss a potential collaboration with Janssen Pharmaceuticals and the Bill and Melinda Gates Foundation in the roll-out of Bedaquiline (drug treatment for TB).
- Site assessments to prepare three additional CTCTC sites (Shenzhen Donghu Hospital, Shenzhen; Changsha Central Hospital, Changsha; and Zhenjiang No.3 People's Hospital, Zhenjiang) to participate in future studies were also conducted.
- FHI 360 also translated the Chinese CTCTC standard operating procedure (SOP) version 1.0 into English. The translated version of the SOP will be posted on the internal Beijing Chest Hospital Clinical Trial's website and will also be shared with non-Chinese sponsors or partners willing to work with the CTCTC Network.

The FHI 360 China-based team is preparing to invite a data management consultant to make a presentation to the CTCTC leadership regarding the importance and requirements for setting up a data management coordinating center.

OTHER INSTITUTES WITHIN THE NIH

Clinical Center

Critical Care Medicine Department

Support Provided by the Clinical Monitoring Research Program

District of Columbia Partnership for HIV/AIDS Progress – Neurocognitive Initiative

The District of Columbia Partnership for HIV/AIDS Progress (DC-PFAP) established a Neurocognitive Initiative in FY2013 to evaluate how human immunodeficiency virus (HIV) affects thinking, memory, and concentration. Neurocognitive disorders range in severity and are a feature of HIV/AIDS despite antiretroviral therapy. The DC-PFAP Neurocognitive Initiative is a multi-institute collaboration involving investigators from the Critical Care Medicine Department, the National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Mental Health (NIMH), National Eye Institute (NEI), and National Institute on Aging (NIA).

The program comprehensively performs both short-term and long-term assessments of neurocognitive function in cohorts of HIV/AIDS patients by combining the perspectives of the various partnering institutes. The ultimate goal of the program is to develop interventions to improve neurocognitive function and/or slow the neurocognitive decline in HIV-infected individuals.

A CMRP protocol nurse coordinator manages the regulatory operations of the protocols supported by the initiative. The protocol nurse coordinator coordinates the participants' multidisciplinary study visits, manages the collection and organization of patient data, communicates results to study participants, and arranges clinical follow-up needs with the primary care or infectious disease physicians.

The CMRP clinical project manager and special projects administrator manage two subcontracts (one on-going, one new). The agreement with Matthews Media Group provides support for patient recruitment to the various clinical studies and patient transportation to NIH. The new agreement with Providence Hospital in Washington, D.C. was established to assist with recruiting patients for neurocognitive studies. The clinical project manager and senior special projects administrator also manage the DC-PFAP Neurocognitive Initiative budget and work closely with the government project lead and Administrative Office to ensure the money is spent effectively.

The Neurocognitive Initiative's first intervention protocol, "Anakinra, a recombinant human IL-1 receptor antagonist for neuroinflammation in HIV-1 infection" (15-N-0183), was approved by the IRB in August 2015. It is a Phase 1 study to determine the safety of using anakinra over eight weeks in patients living with HIV and also the potential to reduce neuroinflammation. The Emmes Corporation will monitor the study, NIH will be the coordinating center, and Johns Hopkins Hospital will be a satellite location. The protocol is now open to enrollment.

DC-PFAP also began seeing HIV-infected patients under the protocol, "Natural History Study of Inflammatory and Infectious Diseases of the Nervous System" (15-N-0125). This protocol allows investigators to study the natural course of infectious and inflammatory diseases of the nervous system and identify the underlying mechanisms that contribute to neurological outcomes. DC-PFAP investigators have utilized this protocol to study HIV-infected participants with neurocognitive issues that do not fit criteria for our other studies.

The pre-award acquisition process to subcontract with Providence Hospital for assistance with recruiting patients for neurocognitive studies occurred during the FY2016 period. Providence Hospital has a busy infectious disease clinic that will ideally be able to refer both HIV-infected and uninfected participants for Neurocognitive Initiative protocols recruiting at NIH. Providence will also provide space and staff to support the DC-PFAP neurologist in seeing patients with neurologic concerns. CMRP is providing ongoing management and oversight of the subcontract with Providence Hospital.

During FY2016, the CMRP financial program manager worked diligently with the Leidos Biomedical Research Finance Office, the government project lead, and the government Administrative Officer to provide accurate and timely estimates at completion for the FY2016 and FY2017 budget years.

The protocol nurse coordinator traveled to Monrovia, Liberia in September and October 2015 as part of the PREVAIL III study team, along with the DC-PFAP neurologist, to provide trainings to the PREVAIL III medical staff and perform neurological and neurocognitive assessments on Ebola virus disease survivors.

Center for Infectious Disease Imaging (CIDI)

The Center for Infectious Disease Imaging (CIDI) is a cooperative initiative between Radiology and Imaging Sciences at the NIH Clinical Center and the National Institute of Allergy and Infectious Diseases (NIAID). CIDI was established to perform basic science, translational, and clinical research on the imaging features of infectious disease in computed tomography (CT), nuclear medicine, magnetic resonance imaging (MRI), ultrasound, radiography, and optical modalities. These efforts are designed to advance the knowledge of radiology-pathology-virology correlation with clinical translation in the study of infectious disease and emerging pathogens. CIDI offers opportunities for intramural NIH and extramural investigators to collaborate on infectious disease research using advanced imaging technologies.

The CMRP imaging scientist works under the CIDI umbrella and supports animal imaging research activities at the NIAID Integrated Research Facility (IRF).

Major CIDI program components to support IRF include:

- Conducting animal model research to investigate pathophysiology, innovative imaging techniques, and develop drugs and vaccines;
- Developing quantitative and computer-assisted–detection methods to assess infectious disease severity and response to therapy; and
- Fostering collaborative partnerships with scientists at the advanced containment laboratories with imaging capability at the IRF campus at Fort Detrick.

In FY2016, the CMRP imaging scientist supported 10 animal model research imaging studies of infectious diseases such as Ebola, Middle Eastern respiratory syndrome (MERS) coronavirus, and Nipah virus. These studies are performed to test the efficacy of putative therapies, vaccines, and/or to establish the virulence of a virus strain. In addition, imaging biomarkers are being developed using CT, MRI, positron emission tomography (PET) (various tracers), and single-photon emission computed tomography (SPECT) to elucidate the effect of the virus and the immune response to internal organ pathogenesis.

The CMRP imaging scientist performed biomarker development for novel CT and MRI measurements of the lung, kidneys, liver, spleen, and brain; developed a workflow to efficiently acquire and process medical images in a robust and reproducible manner; and developed an interface system to schedule imaging exams through the research operations management system that is integrated with IRF medical scanners. Technologists load the animal demographics into the scanner through this interface, acquire images, and send the scans to the research picture archive and communications system (PACS) for long-term storage. Images are retrieved from PACS for quantitative and qualitative evaluation on commercial and in-house custom developed software. A web portal and radiology reporting system was developed to allow the CIDI radiologists to qualitatively assess the imaging scans and report their findings. The CMRP imaging scientist is continually improving the efficiency of all aspects of this workflow.

The radiologists at CIDI at the NIH Clinical Center may provide qualitative interpretations of medical images acquired during infectious disease imaging studies at IRF. To facilitate this effort as well as to improve the communication of findings between CIDI and IRF scientists, a web portal was designed and implemented. This portal interacts with a home-grown image ordering and communication system (irfRIS) and posts a listing of all scans acquired and available for review by the radiologists. The web portal also queries PACS to determine if radiology reports have been created. There are two versions of this portal; one is for radiologists and allows dictation of radiology reports, and the other is used by IRF scientists to view the images and qualitative radiology reports.

At the IRF, the CMRP imaging scientist has established an image analysis suite. This area supports multiple workstations running commercial and research image visualization and analysis software. A relationship has developed with one commercial software vendor that has allowed the program to escalate the development of software tools to meet the research needs of the animal imaging studies of infectious disease. For example, the imaging scientist is collaborating with MIM Software to implement kinetic modeling techniques to analyze dynamic PET and MRI scans (quantify perfusion in organs, uptake of PET tracers, etc.).

Use of the application programming interface called “extensions” extends the commercial product features and is not currently available to other users of this software. For example, we have developed extensions to estimate T1 image maps from dual-flip angle MRI scans. We have also developed R2* maps

from dual-echo MRI scans. We have integrated brain tissue segmentation software that allows us to estimate changes in gray matter volume. These are all serving as biomarker development for diseases such as Ebola. Additional work related to MIM involves supporting multiple licenses and training imaging technologists at IRF to use this software.

Performing quantitative measurements of medical images acquired at IRF, while working with investigators at IRF and radiologists at CIDI, has produced multiple publications; during FY2016, the CMRP imaging scientist co-authored six peer-reviewed abstracts and three peer-reviewed journal publications.

The CMRP imaging scientist supported 10 new imaging studies at IRF. These include five Ebola imaging studies, two MERS coronavirus studies, two Nipah virus studies, and one particle lung deposition study with fluorodeoxyglucose PET, as well as imaging method development studies. Support for these studies included image data workflow, post-processing of images, preparation of analysis reports to investigators, and manuscript development.

Instead of purchasing a commercial radiology information system (RIS) that interacts with scheduling tools and medical imaging scanner equipment, or paying a company to modify the scheduling tools, a custom RIS system (irfRIS) was developed and supported.

National Eye Institute

Support Provided by the Applied and Developmental Research Directorate

Applied and Developmental Research Directorate's (ADRD) Clinical Support Laboratory (CSL) provided ELISA-based cytokine testing of vitreous fluid and eye-wash specimens in support of Dr. Chi Chao Chan, chief, Immunopathology Section, Laboratory of Immunology, and head, Histology Core. Approximately 34 ELISAs were performed, with most requests involving the testing of IL-6 and/or IL-10. Many samples were submitted individually, with requests for rapid turnaround of test results. Additional testing requested included a single request to test 114 samples for IL-17A.

National Institute of Neurological Disorders and Stroke

Support Provided by the Applied and Developmental Research Directorate

Applied and Developmental Research Directorate's (ADRD) Clinical Support Laboratory (CSL) provided sample processing support for Protocol 12-N-0137 under YT13-110. A total of 17 samples were received from eight patients, resulting in the storage of 37 vials of PBMCs. Sample collection is for future ELISPOT testing in the Laboratory of Cell-Mediated Immunity.

The BioProcessing Laboratory provided support to two National Institute of Neurological Disorders and Stroke (NINDS) ancillary Phase II clinical trials:

- Biomarkers in Multiple Sclerosis study. The BioProcessing Laboratory provided specimen lists from the collection belonging to participants with destruction requests. The lab also submitted two proposals for the tracking of participant consent status at the specimen level.
- Biomarkers in Myasthenia Gravis study. The BioProcessing Laboratory participated in end of study data reconciliation and coordinated the relocation of the specimen collection within its freezer unit to the George Washington University.

Support Provided by the Clinical Monitoring Research Program

The National Institute of Neurological Disorders and Stroke (NINDS) conducts and supports research on brain and nervous system disorders, and has occupied a central position in the world of neuroscience for more than 50 years. The mission of NINDS is to reduce the burden of neurological disease by supporting and conducting basic, translational, and clinical research on the normal and diseased nervous system. The institute also fosters the training of investigators in the basic and clinical neurosciences, and seeks better understanding, diagnosis, treatment, and prevention of neurological disorders.

NINDS clinical research applies directly to the mechanisms of nervous system diseases, which can then be translated into disease detection, prevention, and treatment, including studies of brain imaging techniques, trials to test new drugs, and novel therapy development, such as stem cell implants and gene transfer. Some key areas of NINDS clinical research include the neurological consequences of AIDS, Alzheimer's disease, brain tumors, developmental disorders, epilepsy, motor neuron diseases, muscular dystrophies, multiple sclerosis, neurogenetic disorders, pain, Parkinson's disease and other neurodegenerative disorders, sleep disorders, spinal cord injury, stroke, and traumatic brain injury. CMRP provides regulatory support for clinical trial operations at NINDS by serving as liaisons on regulatory issues with sponsors, the U.S. Food and Drug Administration (FDA), the Office for Human Research Protections, and other regulatory bodies; and developing, assembling, submitting, and maintaining Investigational New Drug (IND) applications for the FDA.

The CMRP team supporting NINDS comprises a CMRP senior manager; a CSO director with expertise in protocol document development, clinical research methodology, and Institutional Review Board (IRB) requirements; as well as a regulatory affairs director and an experienced regulatory associate, both thoroughly versed in the process of IND development and submission, and FDA review.

Regulatory Affairs continues to provide support for the NINDS giant axonal neuropathy (GAN) gene therapy team. The team prepared and submitted more than five IND amendments during FY2016. These included two protocol and informed consent amendments; an unanticipated, unrelated adverse event report; a final report of the single-dose toxicity study in rats; updated chemistry, manufacturing, and controls (CMC) information; and a Data and Safety Monitoring Board (DSMB) report.

NINDS requested that a draft version of the amended GAN protocol, which supports escalating the dose of the scAAV9/JeT-GAN, be submitted to the FDA for agency feedback concerning the proposed dose escalation. The investigators proposed an injection interval of eight weeks after the first patient in each dose cohort to monitor for safety, and if continued safety is demonstrated, every six to eight weeks thereafter. In support of the proposed dose escalation, a mouse rotarod performance report and a draft single-dose rat toxicity study were also included in the FDA submission.

Regulatory Affairs prepared and submitted to the FDA the IND annual report that provided a summary of activities with the GAN product covering the period of May 30, 2015 to May 29, 2016.

Regulatory Affairs also worked with NINDS investigators to prepare an expanded access protocol for patients who may not fully qualify for the inclusion criteria in the current GAN clinical study, such as patients who would need additional immuno-modulation and/or have advanced disease. The expanded access, single-patient protocol will be submitted to the existing GAN IND.

Regulatory Affairs prepared an IND amendment containing an unanticipated adverse event: not related (UAE), detailing the death of a study subject as a result of sepsis following elective surgery, but not due to the study agent or participation in this clinical study. The UAE was reviewed by the IRB and DSMB, and the conclusion was that the event was not related and the clinical study could proceed. The IRB and DSMB assessments/reports were also submitted to the FDA once available.

An amendment to the IND CMC information described the change in product manufacturer's name and included a report from the manufacture regarding the out-of-specification (OOS) plasmid level for a recently manufactured lot of VMscGAN. During the investigation of this OOS plasmid level, a calculation error was identified, with the ultimate determination that the limit of residual plasmid DNA was inappropriately set. However, there were insufficient manufacturing data for VMscGAN to support a set limit of residual plasmid DNA, and, thus, the specification for this measurement should have been accepted as the reported value, with no safety concerns expected from the reported levels of residual DNA.

National Institute of Environment Health Sciences

Support Provided by the Applied and Developmental Research Directorate

Applied and Developmental Research Directorate's (ADRD) Clinical Support Laboratory (CSL) provided ongoing support to four studies. Support included preparing and shipping specimen collection kits to trial participants, receiving and processing clinical specimens, extracting DNA, and preparing invoices for National Institute of Environment Health Sciences (NIEHS)–authorized payment of participants and physicians. Multiple specimens were received from approximately 100 patients or family members. In response to YT15-071, the laboratory worked with the Data Management Group to define changes to the patient database necessary to allow NIEHS to enter specimens into the Biological Specimen Inventory system. The database changes were implemented at the end of FY2015.

National Institute of Dental and Craniofacial Research

Support Provided by the Applied and Developmental Research Directorate

In response to CSAS-17612, the Laboratory of Cell-Mediated Immunity performed one set of three- and six-day proliferation assays to evaluate the proliferative response of cells from normal donors against mitogen phytohemagglutinin and a pool of allo-stimulator cells in a mixed lymphocyte culture, in the presence and absence of bone marrow stromal cells at multiple concentrations. A total of 66 tests were performed.

National Institute of Arthritis, Musculoskeletal and Skin Diseases

Support Provided by the Clinical Monitoring Research Program

The mission of the National Institute of Arthritis, Musculoskeletal and Skin Diseases (NIAMS) is to support research that will lead to the promotion of knowledge and understanding of the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases. Toward this effort, the NIAMS Intramural Research Program conducts natural history and treatment studies, as well as basic investigations of the etiology and/or pathophysiology of rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, vasculitis, scleroderma, myositis, osteoarthritis, and other inflammatory/rheumatic diseases.

CMRP provides protocol review services and regulatory and clinical trials management support to allow NIAMS to streamline protocol development time, provide flexibility for emerging/fluctuating needs, eliminate costly time delays, and ensure the success of the clinical mission. CMRP staff supports clinical research operations for NIAMS Investigational New Drug (IND) Phase I and Phase II clinical trials; this support includes writing protocol and informed consent forms, offering regulatory and compliance guidance, training, and clinical trials management. Staff members also assist with document creation, data collection, and compilation for regulatory submissions (pre-IND, IND) to FDA and other regulatory authorities, and technical review and report preparation.

In addition to regulatory and monitoring support, CMRP provides clinical nursing and administrative support. Clinical support includes patient care coordinator staff to assist with scheduling appointments for patients' clinical visits and serve as liaison between physicians, nursing staff, and other departments. CMRP maintains identification and demographic data for each patient, as well as other pertinent patient information required prior to an appointment or admission.

During FY2016, the CMRP Clinical Trials Management (CTM) staff continued to provide NIAMS staff with information on how to best document data in the clinic charts and provided guidance on investigators' responsibilities in overseeing an FDA-regulated IND clinical trial. CTM currently monitors eight active NIAMS studies, including two IND studies, and is responding to a request to test and provide feedback on the new NIAMS electronic regulatory binder.

CTM began to support the following studies: (1) Natural History, Pathogenesis and Outcome of Idiopathic Systemic Vasculitis; (2) Pilot Use of Antiretrovirals in Aicardi Goutieres Syndrome; (3) Safety of Tofacitinib, an Oral Janus Kinase Inhibitor, in Systemic Lupus Erythematosus: A Phase Ib Clinical Trial and Associated Mechanistic Studies (SAFE-JAK-IN-LUPUS), and (4) The Molecular Basis of Primary Immunodeficiency.

In FY2016, the CMRP Regulatory Affairs group prepared and submitted to the FDA a protocol and informed consent amendment for the rilonacept in the deficiency of the interleukin-1 receptor antagonist study (protocol 13-AR-0086; IND 100567), as well as an annual report for IND 100567 that provided enrollment and safety information for the DIRA study.

The annual report for IND 11138, covering the study of anakinra in Behcet's disease (protocol 11-AR-0241), was submitted to the FDA in October 2015. After the protocol was terminated with the NIDDK/NIAMS IRB, the Regulatory Affairs group prepared a final submission to the FDA requesting withdrawal of the IND effective in June 2016.

The Regulatory Affairs group initiated work on two new INDs for NIAMS in FY2016. Both were expanded-access, single-patient INDs to allow treatment with recombinant human Interleukin-18 binding protein (rhIL18-BP; tadekinig-α) for two patients with chronic, uncontrolled auto-inflammatory disease manifested as Macrophage Activation Syndrome (MAS) with interstitial lung disease. Both patients will receive treatment initially as inpatients in the NIH clinical center, and if tolerated, will transition home for ongoing outpatient treatment and monitoring with the collaboration of local physicians.

Regulatory staff offered guidance and advice for preparing these non-standard applications and worked with the principal investigator (PI) to obtain necessary cross-reference authorization and/or chemistry, manufacturing, and controls information from the investigational product manufacturer. The CMRP Regulatory Associate finalized both expanded-access INDs, and submitted the applications to the FDA in summer 2016, allowing the PI to initiate a potential therapy for these patients suffering with a life-threatening disease for which there is no safe, alternative treatment.

The CTM team conducted monitoring visits for the following studies: Natural History and Pathogenesis of Neonatal Onset Multisystem Inflammatory Disease (NOMID), A Pilot Study of Anakinra in Behcet's Disease (BD), Safety and Tolerability of Omalizumab in Patients with Lupus (STOP LUPUS), Studies on the Natural History and Pathogenesis of Spondyloarthritis (SpA), A Pilot Open Label Study of Rilonacept (Arcalyst) in the Deficiency of the Interleukin-1 Receptor Antagonist (DIRA), and the role of PPAR-γ agonists in immunomoduclation and vascular prevention in

Sytemic Lupus Erthematosus (PPAR-SLE). In addition, quality assurance reviews were completed as done in previous years for the Stopping Anti-TNFi Withdrawal in Rheumatoid Arthritis (STARA).

CTM will initiate the following studies for NIAMS in FY2016: Natural History, Pathogenesis and Outcome of Idiopathic Systemic Vasculitis, and a Pilot Use of Antiretrovirals in Aicardi Goutieres Syndrome.

National Institute of Mental Health

Support Provided by the Clinical Monitoring Research Program

CMRP provides protocol development/ navigation, regulatory guidance/support, clinical trials management support, and data and safety monitoring for intramural clinical research protocols being conducted by the National Institute of Mental Health (NIMH).

The CMRP Protocol Navigation Management Program was initiated in March 2014 and consists of 122 protocols, of which 79 are active and 17 are in the data analysis phase. In addition, another 14 protocols are in the review process and could become active in the coming months.

The program activities include managing, tracking, and coordinating associated regulatory activities for each of the 122 protocols. Each protocol requires yearly regulatory oversight in the form of a continuing review (CR), which requires a coordinated effort between the protocol navigation manager (PNM), the medical writer (MW), the research staff, and the Institutional Review Board (IRB). The CRs are deadline-driven and require quick and accurate responses to any IRB-generated tasks prior to and after the CR review. The most time-sensitive work for the NIMH project is making sure the CRs are submitted on time.

To date, the program has improved the CR submission process by helping PIs navigate the new process, which involves using new submission forms, constantly evolving templates, and IRB standard language.

The Protocol Navigation Team (PNT) works with the NIMH PIs and clinical study staff to ensure protocols and informed consent forms (ICFs) are consistent with policies that govern human subject research.

This PNT is also responsible for managing, tracking, and coordinating associated regulatory activities, providing protocol writing support, and providing expert-level regulatory guidance to PIs, key medical staff, and clinical study staff during conception/development and throughout all aspects of a protocol's life cycle, including continuing review applications, bioethics reviews, protocol amendments, scientific reviews, and applicable Data and Safety Monitoring Board (DSMB) reviews.

The PNT assists and liaises with PIs on regulatory issues with the sponsor, the U.S. Food and Drug Administration (FDA), the Office for Human Research Protections (OHRP), and other regulatory bodies as applicable. The PNT serves as a liaison between clinical monitors, clinical research sites/laboratories, regulatory agencies, vendors, and other applicable internal departments. The PNT also provides oversight and support to the PIs for IRB initial reviews, continuing reviews, ICFs, and responses to stipulations, as well as regulatory documentation, including clinical report writing, FDA Investigational Device Exemption/ Investigational New Drug (IDE/IND) serial submissions, safety reporting, and annual FDA reporting.

The PNT continues to add significant value to the protocol development process by providing protocol services related to protocol and consent drafting and logistics management, and assisting new and experienced clinical investigators. The PNT assists PIs in editing clinical protocols in any stage of document development: study concepts, initial review, ICFs, amendments, standard operating procedures, and continuing reviews. During protocol development, the team collaborates with PIs to ensure compliance with regulatory requirements, NIH policies, and project timelines, as well as to enhance the overall accuracy and quality of content.

The PNT has continued to review existing and pending protocols, with assistance from the associate deputy to the clinical director, and has prioritized the workload based on the protocols that are the most time-sensitive and have the greatest need for protocol navigation assistance. The PNT is now assisting PIs with updating all of their protocols with new IRB template language. This change affects all protocols actively enrolling and newly developed protocols that were drafted on older templates.

During the reporting period, 92 continuing reviews have been completed or initiated by the PNT, and by September, 125 continuing reviews will have been initiated or completed. The PNT also assisted with 10 study closures during this reporting period.

The PNM supervises the MW, which requires serving as a project leader and mentor, and providing feedback on employee performance. The PNM also oversees the writer's workload and schedule, as well as reviews the work deliverables for quality, accuracy, and timeliness.

In addition, the PNM is assisting NIMH with an effort to conduct scientific reviews on all protocols within the next three years and to maintain this review schedule for all protocols going forward. The PNM is also assisting the NIMH with updating all protocols so that they contain the appropriate data-sharing language. The PNM serves on the Data Sharing team, which reviews the implementation and execution of data sharing for all applicable protocols.

The PNM attends monthly IRB meetings to gain insight into new and ongoing regulatory issues and to build relationships with the IRB members. The PNM attends two bimonthly meetings with the NIMH team members as applicable, providing updated status reports on clinical/regulatory activities, data quality, and FDA/IRB and DSMB submission timelines. The PNM serves as the executive secretary to the DSMB and provides technical and operational management for all DSMB activities.

The NIMH training database established by the PNM continues to be updated and maintained by the PNT. This database provides a convenient location where all of the NIMH PIs' and associate investigators' (AIs) training information can be located. This database will continue to help the PIs/AIs remain compliant with the training requirements for conducting clinical research studies. In addition, a site on the NIMH SharePoint website has been approved for use by the PNM. The site is still in development and will serve as a repository for all current versions of NIMH protocols and ICFs once complete.

National Heart, Lung, and Blood Institute

Support Provided by the Clinical Monitoring Research Program

CMRP services provided to the National Heart, Lung, and Blood Institute (NHLBI) have resulted in the rapid deployment of clinical services for time-sensitive, critical clinical research. CMRP helped to streamline protocol development time, provide flexibility for fluctuating needs, eliminate costly time delays, and ensure the success of the NHLBI clinical mission. The CMRP staff support to the NHLBI protocol navigation team (PNT) currently consists of a clinical project manager and five protocol navigators who manage 225 clinical protocols and ensure proper submission of all NHLBI clinical protocols to the Institutional Review Board (IRB) and U.S. Food and Drug Administration (FDA), as applicable.

During FY2016, CMRP activities centered on regulatory compliance and protocol navigation services such as data/document collection and compilation for regulatory filing (pre-Investigational New Drug [IND], IND, and Investigational Drug Exemption [IDE]) with FDA and other regulatory authorities; technical review and report preparation; protocol navigation support, administrative coordination, and general logistical support for regulatory activities; and training.

The CMRP clinical trials director continued to have regularly scheduled calls with the government customer, provided monthly metrics and financial data updates, met regularly with the protocol navigation manager to discuss updates, NHLBI strategies, and work distribution.

CMRP staff frequently met with NHLBI principal investigators (PIs) to discuss specific clinical research needs and their unique research and regulatory requirements, and provided PNT's assistance with the development of the investigators' new clinical research program.

To date, the PNT has been instrumental in providing comments to the staff of the NHLBI Office of the Clinical Director (OCD) on the following: Division of Intramural Research (DIR) Clinical Research quality assurance (QA) and quality control (QC) audit review standard operating procedures (SOPs); DIR policies; National Institutes of Health (NIH) policy changes; NHLBI protocol navigation SOPs; and updates on the status of clinical research development plans for multiple research teams.

This last reporting period has seen an increase in the number of reliance agreement request submissions to the NIH Office of Human Subjects Research Protections Program (OHSRP) to facilitate the participation of outside investigators and/or institutions into intramural NHLBI protocols. Two events have contributed to this increase: 1) NIH recently started requiring reliance agreements for the review of identifiable data by investigators working outside of the institute and 2) NHLBI started to use the single IRB model for multicenter collaborations and the NHLBI IRB became the IRB of record for three multicenter studies. CMRP protocol navigators ensure all information is properly addressed in the protocol prior to the agreement request to OHSRP, work with the IRB and support team to review local site consent content, obtain local context information to ensure human subjects protection issues are properly addressed, and collect administrative approvals and data from the sites to ensure regulatory submissions are complete. PNT's involvement with reliance agreements is expected to grow as single IRBs become more common.

Overall services provided by the CMRP protocol navigators in support of the NHLBI Protocol Navigation (PN) Office include: (1) collaborating with PIs and the research team to prepare or assist in the preparation of draft study protocols; (2) writing/ editing sections of IND/IDE submissions, as well as providing information and guidance on the process; (3) reviewing and editing protocol documents to ensure that regulatory requirements are met; (4) working with clinical research teams and data managers to compile data for reports; (5) processing and managing final documents, including uploading documents into different systems, routing documents for signatures, and sending reminders for continuing review documents, protocol/ informed consent form (ICF) amendment documents, and protocol deviations, assisting PIs with responses to IRB stipulations, and addressing unanticipated problems; (6) working with the clinical research teams to submit requests for informed consent short forms and

informed consent translations; (7) organizing branch review of new studies and preparing meeting minutes for the branch chief and clinical director; (8) ensuring that the participation of external investigators and/or collaborating sites in NHLBI protocols are addressed through appropriate agreements; and (9) working with the Deputy Ethics Clearance Office and NIH Ethics Office to ensure "covered" individuals on "covered" protocols undergo proper ethics clearance.

The CMRP clinical project manager supports the NHLBI OCD and PIs by: (1) managing the quality of FDA regulatory submissions generated in the PN Office; (2) serving as a liaison between the PN Office, the Office of Clinical Affairs, and/or OCD; (3) serving as the liaison between the PN Office and the data management and monitoring contractors; (4) providing first-line assistance with clinical protocol design for less-experienced clinical investigators; (5) implementing processes within the PN Office to ensure the quality and uniformity of the documents generated by the protocol navigators; (6) serving as the regulatory manager for a high-profile, multicenter (n=20) IDE study sponsored by NHLBI; and (7) serving as the expert on data entry into the IRB submission database, iRIS, assisting both NHLBI and non-NHLBI investigators with their iRIS submissions.

During this reporting period, the PN Office had some unexpected staffing challenges (i.e., extended medical leave, resignations) that were addressed swiftly to allow the program to continue to successfully deliver high-quality documents to the investigators, the OCD, and the Office of Clinical Affairs (OCA). The clinical project manager temporarily assumed additional responsibilities for processing requests related to a portfolio of studies, distributed several studies among the other navigators, and worked with the OCD and OCA to prioritize submissions until the vacancies were filled.

In order to assess team resources and workload projections for the protocol navigators supporting the NHLBI intramural program, the clinical project manager created a document that detailed the level of expansion the program had undergone in both number of studies and number of protocol amendments. This document also showed the projected continuation of this expansion based on trends for the past three years; NHLBI had a net increase of almost 50 studies during that time. During the past year, the number of amendments submitted by the PNT increased by more than 80 percent.

The NHLBI Cardiovascular and Pulmonary Branch (CPB), which conducts research on diseases that affect the heart, blood vessels, and lungs, requested that Leidos Biomed provide the support of a pulmonary function technologist to perform pulmonary function tests on subjects. In addition to performing this test for clinical trials, CPB performs these tests as a service to other institutes at NIH to check the status of lung diseases, diagnose conditions, check the extent of damage caused by conditions, or check the effectiveness of treatments for pulmonary diseases.

There are currently 22 INDs (including two emergency INDs), five IDEs, and 36 FDA-regulated protocols within these IND/IDEs, which are managed by the NHLBI PN Office. The other 189 NHLBI protocols managed by Leidos Biomed protocol navigators are non-FDA–regulated protocols and range from treatment protocols investigating off-label use of approved agents to natural history studies and training protocols.

CMRP protocol navigators handled the following regulatory (FDA) submissions on behalf of, or in collaboration with, NHLBI investigators: IND submissions (including one emergency IND), amendment submissions for active INDs/IDEs, and IND terminations. The protocol navigators also assisted with: (1) IRB submissions that include 18 initial/new protocols, 204 drafted continuing reviews, 353 protocol amendments, 110 NIH problem report forms (includes deviations and serious adverse events [SAEs]); (2) 28 DMSB reports; (3) six OHSRP exemptions; (4) 11 Reliance Agreement requests to the OHSRP, and (5) 20 meeting minutes providing a summary of new protocol reviews to the branch chief and clinical director. In addition, the PN team performed a QC review of 20 new protocols, 182 IRB continuing reviews, 310 protocol amendments, 100 NIH problem report forms, 20 DSMB reports, and six OHSRP exemptions. There has been a significant increase in the number of protocol amendments due to: (1) two sets of new requirements (two amendments for each protocol) for specific language pertaining to the inclusion of NIH employees as research subjects to be added to certain protocols and consents; (2) new requirements for specific language pertaining to the role of investigators not located at the NIH and the submission of reliance agreement requests to the OHSRP; (3) new NIH policies related to human data sharing and genomic data sharing; and (4) more studies are being activated compared to those studies being closed or moved into data analysis stages.

During FY2016, CMRP assisted with a response to congressional inquiries related to fetal tissue research, a for-cause audit, and corrective action plans. CMRP protocol navigation team members facilitated the successful submission of an amendment, approved by the IRB without stipulations, for the Sickle Cell Branch to establish a protocol that can serve as a future biorepository for the Branch, and also supported the timely closure of 11 clinical studies that had been left in data analysis upon the completion of endpoints.

The PN team worked closely with the OHSRP on many occasions due to the increased requirement that associate investigators from outside institutions have

reliance agreements in place; 11 reliance agreements between the NIH and several different universities were submitted and approved.

CMRP managed a number of multicenter clinical trials for NHLBI in FY2016; one large trial expanded from 11 to 20 sites, another trial increased to five sites, and three studies that were originally single-center studies were turned into multicenter studies. One resulted in a large collaboration with the University of Maryland in Baltimore (UMB) that will expand to other protocols in the future. The clinical project manager participated in a task force to come up with uniform processes for dealing with the increase in multicenter studies that will rely on an NIH IRB (single IRB study).

CMRP supported the submission of all regulatory documentation required to remove the clinical hold from six different NHLBI INDs/IDEs following NIH Pharmaceutical Development Section (PDS) shutdown in June 2015. Five of the six submissions have been taken off hold and one study encountered problems with cell production in the process of responding to the PDS issue; discussions with the FDA on the best way to approach the issue are ongoing.

National Human Genome Research Institute

Support Provided by the Applied and Developmental Research Directorate

Applied and Developmental Research Directorate's (ADRD) Clinical Support Laboratory (CSL) received 34 whole-blood samples from patients enrolled in clinical trials 00-HG-0209 or 14-HG-0038 for density gradient separation to isolate mononuclear cells. Each day that samples were received, a normal donor research sample was also received for processing. Freshly isolated cells were submitted to the Laboratory of Cell-Mediated Immunity to perform real-time proliferation assays on clinical samples from patients with a variety of immunodeficiency disorders, for a total of 1,736 data points. This ongoing support was initiated through YT07-026 from Dr. Fabio Candotti, and continued through CSAS-16726 after Dr. Candotti's departure from NIH.

Three blood samples were received for EBV transformation in response to two CSAS requests from Dr. Daniel Kastner.

National Center for Advancing Translational Sciences

Therapeutics for Rare and Neglected Diseases

Support Provided by the Clinical Research Directorate

The Therapeutics for Rare and Neglected Diseases (TRND) program was created to accelerate new treatments for rare diseases found in limited patient populations and effective treatments for neglected diseases found in larger patient populations. Through collaborations with research partners, TRND aids in moving small-molecule and biologic drug candidates through milestones of preclinical, clinical, and Investigational New Drug (IND) application with the U.S. Food and Drug Administration (FDA).

The Leidos Biomed Clinical Research Directorate (CRD) has supported TRND since 2009 through a broad range of activities and the management of candidate projects. Specifically, the team has provided the following services that support drug candidate development: lead optimization; in vitro pharmacology (absorption, distribution, metabolism, and excretion [ADME]); toxicology testing; biomarker assay development; in vivo drug metabolism and pharmacokinetics (DMPK), toxicology, and disease animal model development; chemistry, manufacturing, and controls of active pharmaceutical ingredients (APIs) and formulated drug products; and regulatory and clinical trial support. In addition, we have provided project and program management support, financial analysis, and subcontract administration for services noted herein.

Leidos Biomed has initiated support for four new development projects based on TRND's established agreements with academic or commercial partners for treatment of the following conditions: malaria, hemoglobinopathies, sickle cell disease, and Zika virus. In addition to the new development projects, efforts are ongoing to advance approximately 16 existing projects in various stages by maintaining subcontracts and procuring additional support services through the development cycle, or completing and closing the projects.

Within the CRD support to the National Center for Advancing Translational Sciences (NCATS), the support team has enhanced support to the TRND program by providing improved service through financial reporting, project planning and tracking, accelerated subcontracting efforts, and process improvements. Furthermore, the team has developed blanket agreement relationships in order to improve the efficiency for critical service needs of in vitro pharmacology and other laboratory services and to accelerate the procurement process.

Support Provided by the Clinical Monitoring Research Program

The U.S. Congress mandated that the National Institutes of Health (NIH) establish the Therapeutics for Rare and Neglected Diseases (TRND) program in 2009. This unique program creates a drug-development pipeline within NIH, and is specifically intended to stimulate research collaborations with academic scientists, nonprofit organizations, and pharmaceutical/biotechnology companies working on the treatment of rare and neglected illnesses. Along with developing new candidate drugs for rare and neglected diseases, TRND seeks to advance the entire field of drug discovery and development by encouraging scientific and technological innovations aimed at improving success rates in the crucial early stages of drug development.

In addition to performing extensive preclinical research, TRND conducts studies in natural history, healthy subjects, and the treatment of patients with rare and neglected diseases.

TRND has numerous active projects, including non-drug, natural history studies (NHS), and Phase I and II patient trials. Currently, two of these protocols are being conducted under Investigational New Drug (IND) applications, and two additional studies will require IND applications prior to approval and initiation.

During FY2016, CMRP provided a variety of services to support four TRND IND studies (two active and two in development), including assembling, reviewing, and submitting IND applications; maintaining IND and regulatory documents; directing communications between TRND, IND sponsors, the U.S. Food and Drug Administration (FDA), and the Institutional Review Board (IRB); developing materials for, and participating in meetings with, the FDA and TRND study teams; providing clinical trials operational support, with quality assurance and quality control oversight; developing protocol and informed consent forms; Good Clinical Practices (GCP) monitoring of clinical trials; providing audit support for regulatory inspections; and training for investigators and site personnel on GCP and records management guidelines.

Regulatory Affairs prepared and submitted a request to add cryptococcal meningitis to the FDA's list of tropical diseases. This request involved preparing background information about the disease and how it fit the FDA's requirements to be classified as a tropical disease. This was the first time Regulatory Affairs and TRND personnel had prepared or submitted such a document, and it involved a great deal of research, both on the format and submission process, as well as with regard to the scientific background.

Regulatory Affairs concluded its work on TRND's Niemann–Pick disease type C team by completing the total transition of this project (including all regulatory files and tasks) from TRND to the new IND sponsor, Vtesse, Inc.

Regulatory Affairs and Clinical Trials Monitoring (CTM) staff also continued to work with the Hereditary Inclusion Body Myopathy (HIBM) project team, which included discussions of extending the Phase I and ongoing Phase II trials, and developing the IND annual report. The Regulatory Affairs staff drafted the IND annual report and worked with the HIBM study team to compile the data, review the content, and complete the document for submission in October 2015. Regulatory Affairs staff also initiated the transfer of this IND to its new sponsor, Fortress Biotech. This transition is ongoing and anticipated to be completed by the end of FY2016 or early FY2017.

Following activation of the HIBM Phase II study, the CTM team performed two monitoring visits in FY2016, after which CTM worked with the study team to address source document discrepancies and other minor items noted during these visits. The CTM team conducted the third monitoring visit in April 2016 to ensure the study was monitored according to the plan. Based on the study timeline, the protocol follow-up has been completed, and CTM is working with the new IND holder and the NCATS principal investigator to assess how many more monitoring visits will occur and the timing for the study close-out visit in FY2017.

Regulatory Affairs assisted the TRND Hypoparathyroidism team, with work focused on project development and plans to request a pre-IND meeting with the FDA. Regulatory Affairs provided regulatory guidance for team members who were unfamiliar with the pre-IND process and participated in monthly teleconferences regarding updates on the hypoparathyroidism project.

Regulatory Affairs also continued its work on TRND's development of ceramidase for Farber's disease, in conjunction with the company Plexcera. Regulatory Affairs facilitated interactions with, and made submissions to, the FDA regarding the project's development.

Regulatory Affairs also began participating in teleconferences with the NCATS project manager and the pharmaceutical developer for a new project evaluating benserazide for the treatment of hemoglobinopathies. Initial regulatory support for this project involved the development and submission of a pre-IND meeting request letter and information package, with FDA submission to occur in the summer of 2016. The timeline for IND preparation and submission will be determined based on the results of the pre-IND meeting with the FDA.

Bridging Interventional Development Gaps

Support Provided by the Clinical Research Directorate

The Bridging Interventional Development Gaps (BrIDGs) program was created to provide critical resources for developing therapeutics for common and rare diseases. Through collaborations with research partners, BrIDGs provides crucial services related to synthesis, formulation, pharmacokinetics, and toxicology that will support the partner's Investigational New Drug (IND) applications with the U.S. Food and Drug Administration (FDA).

Leidos Biomed has supported BrIDGs since 2009 through the services noted here and the management of candidate projects. The team has provided the following services that support drug candidate development: chemistry, manufacturing, and controls of active pharmaceutical ingredient (API) and formulated drug products, pharmacokinetic and absorption, distribution, metabolism, and excretion (ADME) studies, and toxicology studies.

Leidos Biomed has initiated support for three new development projects, based on BrIDGs' partnerships, for the treatment of chronic pain, drug abuse, and depression. In addition to the new development projects, efforts are ongoing to advance approximately 20 existing projects by maintaining subcontracts and procuring additional support services through the development cycle, or completing and closing the projects.

The team from Leidos Biomed's Biopharmaceutical Development Program (BDP) completed tech transfer and scale-up of the development and optimization of a complex recombinant human protein. This project leveraged the BDP's resources to produce the bulk drug and improve its purity to the level that is acceptable to the FDA, in order to continue with toxicology and clinical studies. Recent additional responsibilities include conducting a clinical study to characterize the safety and tolerability of cannabidiol in order to determine whether it should remain scheduled under the Controlled Substances Act or be recommended for decontrol by the National Institute on Drug Abuse, and providing full development support of a metabolite (new molecular entity) for the treatment of major depression (National Center for Advancing Translational Sciences [NCATS]).

OTHER AGENCIES

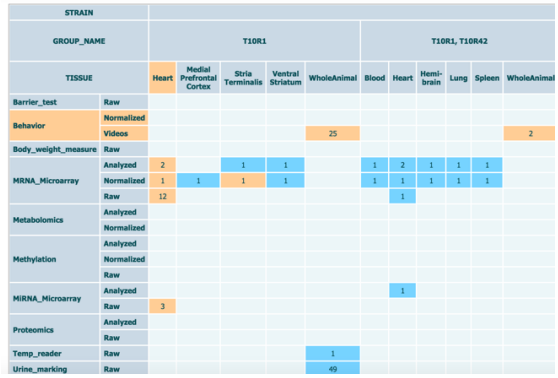
U.S. Army Center for Environmental Health Research

Support Provided by the Data Science and Information Technology Program

The Core Infrastructure and Systems Biology Group (CISB) in ABCC supports the analysis and systems biology efforts at the U.S. Army Center for Environmental Health Research (USACEHR). The support is provided under two major projects, one of which is for providing omics analysis and algorithm development support while the other is to create a systems biology data cube (SysBioCube) that will serve as a central portal for data collection, integration, analysis, mining, and knowledge sharing by army, academic, and private institution collaborators.

As part of the analysis project, CISB has provided extensive analysis support for microarray, methylation, RNA-Seq, miRNA-Seq, and metagenomics analysis for several research collaborations on the diseases of military relevance. The group has created sequencing pipelines and has also contributed to multiple manuscripts and posters on post-traumatic stress disorder (PTSD), sequencing pipelines, and analysis workflows.

The complex analysis in the systems biology collaborations often involve development of new statistical algorithms and visualization packages. In the past year, CISB has developed Panoromics and Heat Cube, which are data-driven visualization packages that can be used for custom visualization of analysis results in studies such as longitudinal studies on complex diseases and multi-tissue drug response.



STRAIN												
GROUP_NAME		T10R1					T10R1, T10R42					
TISSUE		Heart	Medial Prefrontal Cortex	Stria Terminalis	Ventral Striatum	WholeAnimal	Blood	Heart	Heml-brain	Lung	Spleen	WholeAnimal
Barrier_test	Raw											
Behavior	Normalized											
	Videos					25						2
Body_weight_measure	Raw											
MRNA_Microarray	Analyzed	2		1	1		1	2	1	1	1	
	Normalized	1	1	1	1		1	1	1	1	1	
	Raw	12						1				
Metabolomics	Analyzed											
	Normalized											
Methylation	Analyzed											
	Normalized											
	Raw											
MiRNA_Microarray	Analyzed							1				
	Raw	3										
Proteomics	Analyzed											
	Raw											
Temp_reader	Raw					1						
Urine_marking	Raw					49						

Screenshots from the development versions of Panoromics and Heat Cube, which help visualize biological changes across patient cohorts, time-points, and tissues.

For the SysBioCube project, CISB created a new upload and download interface. The new interface alleviated all the security concerns with the Java Applet in the previous interface. The new interface also provides a disease- and project-agnostic single page view of all the available files, where users can select by the table cell or row and column headers in different levels. The downloaded files maintain the operating system (OS) directory structure, thereby avoiding any confusion in complex studies with multiple species and cohorts.

A new sample tracker tool was created to track samples across time points and different patient cohorts. The tool provides an easy-to-use interactive method to assess patient numbers and samples, thereby providing researchers valuable information on the actual numbers available for analysis as well as allowing them to strategize for the next set of sample collections.

Operational Support

Leidos Biomedical Research, Inc.

OPERATIONAL SUPPORT

ENVIRONMENT, HEALTH, AND SAFETY

Safety and Environmental Management

The Environment, Health, and Safety Directorate (EHS) continued efforts to improve numerous Occupational Safety and Health Administration (OSHA)-related safety programs and collaborate with organizations to enhance conditions that contribute to a safe and healthful workplace. Transformative changes made in the last year include the establishment of user committees to engage stakeholder involvement in safety; development of a new, web-based safety inspection and issue management system to transform the way safety deficiencies are tracked to closure and make the process transparent to all stakeholders; new construction safety, confined space, and control of hazardous energy control programs; and streamlining of numerous processes. Assessments began to allow for collection of data to be used to proactively manage safety in laboratories. This management system allows for increased responsiveness and timeliness to meet customer needs.

To promote safety communication at NCI at Frederick, EHS hosted a small vendor fair, with an emphasis on ergonomics. Also, the third nomination for the NCI at Frederick Champion of Safety was revealed in June. The goal of this program is to raise awareness and promote a culture of safety by showing NCI at Frederick staff members at work in their respective workplaces. To illustrate changes in the policy, a poster campaign was launched to promote the new minimum attire and personal protective equipment (PPE) initiative. EHS has also standardized communications using the NCI at Frederick Communication Center tools.

EHS continues to work with the NCI at Frederick webmaster to update and improve the EHS web pages for a more cohesive appearance that will facilitate easier navigation. A major initiative to revise the current EHS compliance manual into procedures was undertaken with subcontractor The Redstone Group. Redstone is working with the EHS subject matter experts to develop more robust and clear procedures.

EHS has launched a new annual safety refresher training class for deployment to all NCI at Frederick employees. The objectives of the training are to ensure that all employees know how to identify hazards in the workplace, protect themselves by reducing or eliminating potential hazards, protect themselves in an emergency, and properly dispose of hazardous wastes. The objectives also include ensuring that all supervisors know their responsibilities and communicate "what's new in safety" to the NCI at Frederick community. EHS worked closely with Data Management Services (DMS) to host this training class online at the NCI at Frederick training portal. Feedback from the training was gathered in Survey Monkey, most of which was positive, and the constructive criticism will be used to improve the training next year.

Annual Facility Safety Inspections

To monitor compliance with NCI at Frederick–approved safety and environmental requirements, EHS has the authority to enter all areas/facilities to make periodic, routine, or unannounced safety inspections. One of the mechanisms employed to satisfy this contractual agreement is the performance of facility-wide safety inspections.

Results of initial inspections are reported to the laboratory chief/manager/program head for corrective action. An attempt is made to resolve all deficiencies in safety and environmental regulations through the appropriate lines of authority within 45 days.

In cases where deficiencies are not resolved within 45 days, the next level of authority provides assistance to EHS to aid in deficiency resolution. EHS is contractually obligated to immediately report any safety inspection deficiencies that are unable to be resolved within 45 days to NCI at Frederick.

As of July 20, 2016, 818 safety-related deficiencies were found this year, facility wide. Of these, 748 deficiencies have been closed. EHS is currently working with the appropriate individuals to close the remaining 70 open deficiencies. Deficiencies not resolved within the initial 45 days were reported to NCI at Frederick.

During 2016, EHS continued to work closely with DMS in the development of several new components of the web-based Safety Inspection and Issues Management System (SIIMS). One of the most exciting components is the newly added Assessment Section of SIIMS, which allows for the performance of risk-assessments that are used to create a "snap-shot" of a given lab to include such items as the hazardous chemicals used, the types of work performed within the space, and the safety systems employed to ensure a safe working environment.

Radiation Safety

During CY2016, the Radiation Safety Office provided radiation safety support to approximately 400 radiation workers, 48 open source programs, 6 X-ray programs, 2 gamma-cell irradiator programs, and 1 electron microscope program at NCI at Frederick.

In addition, during CY2016, the Radiation Safety Office provided radiation safety support to approximately 60 individuals, 3 open source programs, 1 X-ray program and 1 electron microscope program at the Advanced Technology Research Facility (ATRF).

On March 23 and 24, 2016 the Nuclear Regulatory Commission (NRC) inspected the NCI at Frederick Broad Scope Radioactive Materials License for Title 10, Part 37 of the *Code of Federal Regulations* (10 CFR 37) Security compliance. The two-day inspection resulted in a clean inspection report with no violations. NCI at Frederick is currently on a three-year inspection cycle.

Regulatory-driven interlock challenges were performed on both gamma-cell irradiators. The interlocks performed as required.

The annual requirement to update our radioactive materials in quantities of concern (RAMQC) information into the National Source Tracking System was completed.

To satisfy federal regulation of 10 CFR 37 (Physical Protection of Category 1 and Category 2 Quantities of Radioactive Material), the NRC licensees must perform and document the following on an annual basis:

- Review of written Access Authorization Program
- Review of Physical Security Program
- Completion of refresher training for authorized users
- Coordination with the licensee's local law enforcement agencies

The NCI at Frederick Radiation Safety Office initiated the performance and documentation of all of the above in February 2016.

Regulatory-driven sealed-source leak tests were performed on all facility sealed sources that require leak testing. No leaks were detected.

Six-month Radioactive Material Inventory questionnaires were forwarded to facility radioisotope programs. The information provided back to Radiation Safety was complete and accurate. A subsequent physical inspection showed that the storage of licensed, radioactive materials was compliant and that these materials were secure against unauthorized removal.

An amendment to add radioactive laboratory space for Dr. Walters' swing space at the ATRF was submitted to the Maryland Department of the Environment (MDE) on March 11, 2016 and approved on March 21, 2016.

An amendment to add radioactive laboratory space for Dr. Morrison's swing space at the ATRF was submitted to the MDE on June 24, 2016 and is still pending approval.

The Radiation Safety Office assisted in the packaging and shipment of 50 radiopharmaceutical drugs from NCI at Frederick to the NIH for Phase 0–Phase I clinical trials from September 27, 2015, to July 18, 2016.

The 2016 round of radiation-producing machine audits (performed by RSO, Inc.) will be completed in September. Eighteen systems (X-ray generators, X-ray irradiators, SPECT/PET CTs, as well as electron microscopes) located at NCI at Frederick and at the ATRF fall under the scope of these audits. No items of noncompliance were indicated during the CY2015 audits.

10 CFR 29 (for NCI at Frederick) and 26.12.10.01 of the *Code of Maryland Regulations* (for the ATRF) mandate that opening procedures for radioactive materials be performed and documented within three hours of receipt. As of July 18, 2016, the Radiation Safety Office met this requirement 100 percent of the time, for both NCI at Frederick and the ATRF.

From September 27, 2015 through July 18, 2016, the Radiation Safety Office processed 425 incoming radioactive material shipments received at NCI at Frederick and three incoming radioactive material shipments received at the ATRF, in accordance with the above-mentioned regulatory requirements.

The Radiation Safety Office performed 100 percent of regulatory-driven contamination surveys each month. Approximately 900 survey samples (with approximately 0.2 percent showing contaminated sites above the NRC action-level requirement of 500 disintegrations per minute [dpm]) were taken each month at NCI at Frederick, and approximately 48 samples (with 0 contaminated sites above the MDE action-level requirement of 220 dpm) were taken each month at the ATRF. This low overall level of radioactive contamination demonstrates that facility radiation workers are keeping exposures to radioactive materials as low as reasonably achievable (ALARA).

All (100 percent) individuals scheduled for bioassays (urine and thyroid) obtained their scans.

The Radiation Safety Office calibrated approximately 175 radiation survey meters in accordance with established, license-driven procedures.

Twenty-two new radiation workers received NRC-mandated new-user radiation safety training to work with radioactive materials (RAM) at NCI at Frederick; two new radiation workers received MDE-mandated new-user radiation safety training to work with RAM at the ATRF.

All (100 percent) approved radiation workers in need of radiation safety refresher training completed the required training.

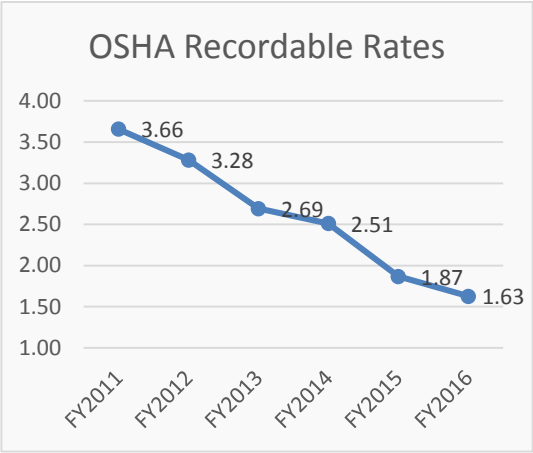
The NCI at Frederick/ATRF Radiation Safety Committee continues to meet regularly to help ensure that all sources of ionizing radiation at the facility are used safely and in a manner that complies with all applicable regulatory and license requirements. The committee reports to the president, Leidos Biomedical Research, Inc. (Leidos Biomed), and provides policy guidance to the Radiation Safety Office.

Non-Ionizing Radiation: The Radiation Safety Office developed a written Magnetic Safety Program and assessed 13 superconducting units.

During CY2016, the Radiation Safety Office provided laser safety support to eight established Laser Programs and 24 individuals. In addition, the Radiation Safety Office established two new Laser Programs and assisted four individuals.

Industrial Hygiene

Industrial Hygiene Injury and Illness Investigations: EHS investigated more than 100 reported work-related injuries, illnesses, and near misses. All responses to Occupational Health Services (OHS) are recorded in the EHS SIIMS database, and reports of all incidents are also generated from SIIMS. The total OSHA recordable injury rate continued to decline in this contract year.



A review of incident trends revealed a lack of administrative and engineering lock out/tag out (LOTO) controls in existing animal facility cage/rack washers. Operating procedures and preventive maintenance schedules have been revised to prevent future incidents. New installations are being engineered with LOTO capabilities.

The PPE policy for laboratories was updated to reflect more stringent minimal clothing requirements promulgated by NIH. EHS collaborated with the Division of Occupational Health and Safety (DOHS) and produced two posters on the topics of laboratory PPE and acceptable lab footwear. In addition, a promotional campaign to educate the campus on the newer requirements was begun utilizing several mediums and communications. The facility incidence where PPE was a causal factor has continued to decline slightly, and only nine cases were documented for the fiscal year.

Environmental/Chemical Monitoring and Risk Assessments: Approximately 1,300 EHS Medical Surveillance Enrollment Forms (MSEF) were reviewed and approved by the Industrial Hygiene (IH), Biological Safety, and Radiation Safety offices. Employees were enrolled in surveillance programs as needed and as indicated. EHS maintains and calibrates 21 individual pieces of monitoring equipment in order to facilitate the timely monitoring of NCI at Frederick needs. A PortaSens II leak detector with formaldehyde monitoring capability was procured to determine peak exposures of personnel working with formaldehyde to more effectively comply with CFR 1910.1048.

EHS contracted with a safety consultant (Redstone Group) to assist with an NCI at Frederick–wide chemical use assessment survey in order to begin the process of accomplishing the following three ultimate endpoints: help streamline laboratory moves and replace the incomplete safety questionnaire with a database system, automate the generation of laboratory door signage, and provide a chemical inventory list in order to provide information on high hazard chemical exposure, use, and storage. The assessment also served as the laboratory program's formal safety inspection for the year. As such, 16 imminent safety deficiencies were cited and immediately corrected, thereby reducing potential risk and ensuring safety. Assessments were completed for approximately 500 individual laboratory spaces during the year. Four high risk laboratories were identified and brought into compliance and acceptable risk by a combination of EHS efforts including: reducing surplus stockpiling of hazardous chemicals, proper storage of flammables, employee training, and PPE usage. A guidance document for a potentially explosive chemical was developed in response to assessment findings, and existing hazards were mitigated.

Additionally, an added functionality of the assessments module was developed to create and print laboratory door signs. This will allow scientific staff and EHS to better ensure the accuracy of door signage as it is aligned with inventory of actual hazards and equipment in the lab spaces.

EHS also developed a basic IH module in SIIMS in order to capture and centralize exposure monitoring data. The module will document and allow reporting by area as well as by individual.

Personal sampling for noise, formaldehyde, isoflurane, methylene chloride, asbestos, and lead was conducted on 25 employees to ensure compliance

with OSHA, American Conference of Governmental Industrial Hygienists (ACGIH), National Institute for Occupational Safety and Health (NIOSH), and NIH policy exposure limits as appropriate.

Eight risk assessment reports for other various work processes were also completed.

Fourteen comprehensive indoor air quality investigations were completed and reported, with corrective and remedial actions included.

Asbestos and Lead Programs: Two EHS staff members are Maryland-licensed asbestos inspectors. EHS assisted Facilities Maintenance and Engineering (FME) in plans that successfully remediated asbestos hazards in Buildings 361, 469 west attic, and 325 in a safe and compliant manner, addressing longstanding risks associated with these asbestos-regulated areas.

EHS conducted eleven asbestos survey reports and one personal asbestos sample survey in support of ongoing FME renovation projects to ensure compliance and worker safety.

EHS trained 98 employees on asbestos and lead paint awareness utilizing in-house resources.

Respiratory Protection Program: There are 334 employees enrolled in the Respiratory Protection Program who require annual fit testing and training. Approximately 87 employees were newly enrolled, while 67 employees were removed from the program.

Hearing Conservation Program: IH conducted 10 noise surveys and eight employee dosimetry tests in various areas throughout NCI at Frederick, including several of the FME mechanical spaces and chiller rooms. Classroom training was conducted for 61 enrollees. Ten employees were added to the program, and 12 were removed.

Forklift Program: Approximately 68 employees are enrolled as forklift drivers; 11 new enrollees were trained and evaluated by EHS, and 12 employees were removed from the program. Tri-annual forklift evaluations were completed for 14 drivers.

Ergonomics: IH conducted approximately 26 ergonomic worksite evaluations and consultations to improve employees' worksites (including office, laboratory, and animal production areas), with the intent of reducing occupational ergonomics-related injuries.

Move Tracking and Renovation Support: EHS efforts to support the refurbishment initiatives resulted in a reorganization and dedication of additional safety staff solely to the design and construction phases of the projects. Efforts to improve the design and project oversight processes and better integrate with FME are underway. EHS created a laboratory Move module within SIIMS to track and relocate laboratory spaces. This move-tracking database, which utilizes the information captured in assessments, has the potential to be a useful tool to streamline the capabilities of other organizations including Property (ALS), FME design, and FME maintenance, as well as a planning tool for scientific programs to allocate resources by space.

Ventilation: EHS performed comprehensive risk-based ventilation studies on two unique custom laboratory enclosures in support of renovation/ refurbishment activities. EHS was able to collaborate with FME in order to create a plan and specification for similar future applications while ensuring continued safety. In addition, EHS was responsible for shepherding projects in Building 434 and Building 536 repositories in order to remove longstanding oxygen deprivation risks and alarming issues.

Mold and Indoor Air Quality: EHS finalized a facility procedure for mitigating mold and water intrusion issues in order to reduce risk of long-term property damage and health impacts due to water leaks and mold. Additionally, 14 comprehensive indoor air quality studies were conducted to evaluate discovered issues. Inspections revealed large issues with three buildings where projects to correct infrastructure issues were initiated.

Environmental Protection

Environmental Protection and Waste Management (EPWM) manages NCI at Frederick's chemical and radioactive wastes, responds to chemical spills or emergencies, tracks emissions of air pollutants, ensures storm water and sediment control compliance, oversees trash and recycling efforts as well as compliance with city sewer discharge permits, conducts training, oversees the facility's Environmental Management System, assists in emergency operations planning, conducts inspections of laboratories, mechanical shops, and warehouses, and is the lead on National Environmental Policy Act (NEPA) programs.

EPWM also lead EHS' efforts towards the facility-wide Energy Conservation Measures. They cleared laboratories, moved equipment, coordinated schedules for the installation of HVAC valves, controls, LED lighting and low-flow plumbing fixtures in laboratories, offices, animal areas, and warehouses.

EPWM submitted NCI at Frederick's annual toxic release inventory, a listing of chemicals used by non-laboratory programs in excess of threshold amounts.

The EPWM staff submits the annual Tier II report, which includes a listing of hazardous chemicals stored in threshold amounts at the facility. This information is sent to the Fort Detrick fire chief, the local municipal fire department, and the Local Emergency Planning Committee (LEPC), as well as the MDE, for emergency planning.

Waste Management

EPWM collects, stores, and ships radioactive and chemical wastes from laboratories and shops at NCI at Frederick, the ATRF, and the Vaccine Clinical

Materials Program (VCMP). It also oversees collection of biohazardous waste by the U.S. Army Garrison (USAG), Fort Detrick, and responds to incidents involving improper waste disposal at the USAG incinerator.

EPWM offers training and investigates incidents that may negatively affect the environment or our customer's compliance record. EPWM conducts inspections of laboratories and shops that use hazardous chemicals and ensures that practices are compliant with current policy.

EPWM assisted in multiple laboratory moves and cleanouts and delivered more than $7,516 in chemicals through its web-based Surplus Chemicals program.

EPWM performed over 94 annual laboratory inspections, including inspections of satellite hazardous waste accumulation sites and off-post facilities. EPWM also hosts training of FME and custodial personnel; provides chemical spill response and oversight of photographic chemical recovery equipment; samples and publishes data on drinking water quality; trains new employees during their orientation; and inspects and maintains Buildings 1067, 1068, and 1071, as well as all spill response equipment and protective gear.

A summary of EPWM's waste collection and disposal efforts for NCI at Frederick from October 1, 2015 through July 20, 2016 appears in the tables below.

Our program continues to be as proactive as possible in reducing waste disposal costs without jeopardizing our many regulatory obligations or hampering the mission of the research and support personnel.

Table I. Hazardous Waste Management October 1, 2015 to July 20, 2016

Waste Type	Waste Quantity
Scintillation vials (nonradioactive)	1,045 liters
Non-halogenated solvents	11,913 liters
Halogenated solvents	12,749 liters
Laboratory packages and miscellaneous	241 containers
Mixed radioactive/chemicals	0 liters

Table II. FNLCR Waste Management Cost Savings October 1, 2015 to June 20, 2016

Material	Quantity Diverted	Estimated Savings
Neutralization	1,188 Liters	$4,645
Surplus chemicals	52 Containers	$4,037
Batteries	5,889 Liters	$9,128

Biological Safety

The Biological Safety staff performs a wide range of safety- and health-related functions to ensure facilities, equipment, and procedures are appropriately evaluated and implemented to provide employee and environmental protection while working within optimum biocontainment conditions at NCI at Frederick facilities.

Institutional Biosafety Committee: The NCI at Frederick Institutional Biosafety Committee (IBC) continues to register all work with human and animal pathogens, recombinant and synthetic DNA and RNA, human cell lines, genetically modified animals, and other potentially infectious materials, including human and animal tissues as well as nonhuman primate materials.

The IBC reviewed and approved 300 registration documents, including new, amendment, and renewal requests.

Thirty-three existing and out-of-date IBC registrations were inactivated.

Forty-seven IBC renewal submissions were approved.

An IBC strain database continues to be maintained as a resource to provide investigators with information on animal strains maintained and research performed on the NCI at Frederick campus.

The IBC monthly meeting minutes are made publicly available at http://web.ncifcrf.gov/ehs/ibc/Minutes.aspx.

The IBC continues to offer training for employees who work with viral vectors. Biological Safety schedules this two-hour class on an as-needed basis;146 employees completed this Viral Vector Safety training.

The IBC, EHS, and DMS completed the development of an electronic IBC registration process. During this reporting period, the electronic system now accepts amendments to IBC registries initially filed through the electronic system. Phase 2 involves the development of a database and information tracking system. The electronic registration process has been well received by both principal investigators (PIs) as well as reviewers on the IBC. Requests for modifications to the registration system have been implemented as we continue to work with investigators to make the transition to the new system as smooth as possible.

Animal Safety: The biological safety officer serves as the Animal Care and Use Committee (ACUC) EHS member, and the biological safety team continues to support additional tasks and responsibilities related to ACUC Animal Study Protocol (ASP) reviews.

EHS continues to research, create, and update detailed Chemical Safety Practices Recommendations (CSPR) reports, which include recommendations for PPE, bedding and feed disposal, special precautions, and engineering controls in support of ACUC protocols and occupational safety. Overall, approximately 135 chemicals/drugs/toxins have been researched for the purposes of performing a risk assessment with respect to animal protocols.

To enhance the ACUC ASP review process, the hazard assessment section of the ASP form was revised to capture additional relevant details in an organized manner. Over the 12-month period, 418 ACUC documents were reviewed to evaluate chemical and biological hazards associated with animal research.

An animal exposure program was developed to address potential and known employee exposures to animal allergens. The written animal exposure program was drafted, and a computer-based assessment tool was developed and implemented on February 24, 2016 to identify employees eligible for enrollment based on their potential for exposure to animal allergens. Currently, 672 employees are enrolled in the animal exposure program. A live training session on animal allergens and the animal exposure program was presented to 40 individuals, and 605 employees completed the computer-based training.

As a result of the 2014 AAALAC inspection, EHS has been tasked with initiatives to clarify animal biosafety containment level requirements in research laboratories and animal housing rooms, update door signage procedures, inventory biosafety containment levels by building, and update the Biosafety in Microbiological and Biomedical Laboratories (BMBL) matrix to include additional hazards and mitigation strategies to minimize personnel exposures. In addition, Biological Safety also provided animal biocontainment training to 150 employees on BSL1, BSL2, ABSL1, and ABSL2 containment practices in response to suggestions for improvement from AAALAC.

Controlled Substances: Biological Safety provides program management and oversight for the Drug Enforcement Administration (DEA) Controlled Substances Program. Biological Safety staff has responsibility for maintaining permits and making applications for new permits. At the present time, both state and federal permits are maintained for both NCI at Frederick and the ATRF. The biosafety staff approves purchases, performs random audits, delivers drugs, and ensures compliance with all applicable DEA controlled substance requirements. Currently, there are 21 active DEA Controlled Substance logbooks.

Tax-Free Alcohol: Biological Safety provides program management for the use of tax-free alcohol within laboratories. Biological Safety staff has responsibility for maintaining permits and making applications for new permits. At the present time, both state and federal permits are maintained for both NCI at Frederick and the ATRF and VCMP. The program includes a semi-annual physical inventory audit. There are currently 125 active Tax-Free Alcohol logbooks.

Autoclave Monitoring Program: Biological Safety continues to intermittently monitor the 107 operational autoclaves on campus and at ATRF. To ensure proper use and safe practices with both shared and dedicated autoclaves, Biological Safety is also developing an autoclave training program.

Shipping Classification/Import and Export Control: Biological Safety is responsible for reviewing all shipment requests and classifying both hazardous and nonhazardous shipments for the Contracts and Acquisitions Directorate, as well as for processing Return of Goods request forms. Biological Safety averages approximately 829 online shipment classifications per month. The Biological Safety staff continues to provide support on import and export control matters and provides assistance with U.S. Customs to obtain necessary permits for USDA/CDC/U.S. Fish and Wildlife Service, etc., on an as-needed basis.

As a result of the addition of several international laboratory sites related to activities for Ebola clinical trials, EHS averages 123 shipment order reviews per month, with some month's reviews nearing 200. These numbers reflect an approximate 70 percent increase in reviews performed on a monthly basis to determine hazard classifications, permits, export requirements, and packaging and labeling requirements as well.

Decontamination: Biological Safety staff assisted with conducting work authorization/laboratory equipment decontamination qualified training. The staff decontaminated an average of 37 biological safety cabinets every month.

Publications: Biological Safety staff continues to update BioMaterial Fact Sheets and Biological Safety Technical Bulletins. These are provided to laboratory and animal care staff to assist with providing minimum practices guidelines for particular agents and biological safety topics, respectively. The following is a list of existing fact sheets and technical bulletins, which were updated during this reporting period:

- Hazards of Chlorine Bleach
- Cold Room Storage
- Sharps use and disposal

Training: The Biological Safety staff offers and coordinates various training sessions for both contractor and government employees.

Werner H. Kirsten Student Intern Program: EHS successfully coordinated the safety training to include hands-on demonstrations, and reviewed and approved the training plan risk assessments for 55 students hired for the 2016 cycle of the

Werner H. Kirsten Student Intern Program. EHS staff worked closely with the mentors and government program administrators in order to enable the students to perform basic science experiments in a way that ensured student safety as well as compliance with OSHA regulations and the applicable NIH policies (including NIH Policy Manual 3015 for minors). In addition, EHS presented the Student Intern Mentor training four times, telecasting three of those sessions to the ATRF. A total of 56 mentors and supervisors attended.

Bloodborne pathogens: Biological Safety staff conducts bloodborne pathogen (BBP) training as part of the new employee safety orientation. Providing this course also maintains compliance with the OSHA requirement to conduct an annual BBP refresher course. Throughout the reporting period, BBP training compliance ranged from 95 to 97 percent, and as of July 2016, nearly 1,267 employees were enrolled. The Biological Safety office worked with OHS to revise the Exposure Control Plan as part of the annual requirements for the OSHA Bloodborne Pathogens standard. Also, Biological Safety staff conducted its annual BBP training session for 39 FME service workers.

Industrial and Maintenance Safety

EHS began several major initiatives to improve the safety and compliance of construction and maintenance–related activities throughout the scope of contract activities. A new safety officer was hired to assist in championing several of these new initiatives and improvements.

Confined-Space Program: EHS began a campaign to label confined spaces in the inventory. Eight Hundred and ninety-six confined spaces were labeled to ensure compliance and manage risk. In addition, training was conducted for 67 facilities personnel on the revised requirements and procedures for entry. EHS has labeled approximately 700 permit required confined spaces and work on creating specific entry procedures is ongoing.

Electrical Safety and Lock-Out/Tag-Out: EHS trained 104 individuals on the newly augmented lock-out/tag-out (LOTO) program. A standard for documentation was refined and implemented and compliance has begun to correct longstanding inconsistencies. EHS continues to work with FME and laboratory programs on the development and implementation of complex LOTO procedures.

Elevated Work and Fall Protection: EHS continues to work with FME on the creation of fall protection plans and elimination of hazards. Plans for permanent anchorage points to support ongoing maintenance are being included in the upcoming respective renovations.

Machine Guarding: EHS contracted with Redstone Group in order to assess the FME maintenance areas and identify shortcomings in fixed facility equipment guards. Approximately 1,800 individual pieces of equipment were evaluated, and 15 were flagged for immediate corrective action due to imminent hazards. The remaining equipment that have shortcomings were labeled with a warning sticker, and FME was trained on recognition of these conditions until future projects to increase compliance are initiated. In addition to equipment located in the mechanical areas, Redstone has assessed machinery located in the FME shops in an effort to bring them up to compliance. Based on their evaluation, a plan for upgrading the existing carpentry shop planer was created in order for FME management to make decisions on future capability with this equipment.

Machine guarding specifications were revised for construction contracts in order to ensure newly installed equipment is compliant.

Construction Safety

EHS has implemented several major efforts to improve the safety and compliance of construction-related activities throughout the scope of contract activities. A construction safety officer has been assigned to lead these efforts. Significant improvements to construction safety oversight were instituted via updated contractual language, procedure creation, communication, training, and workflow/process improvements. These enhancements should result in markedly lower risk at a time when future exponential construction growth is projected/planned.

Construction Subcontractor Safety Program: A robust set of procedures has been developed for Leidos Biomed personnel to help ensure that construction subcontractor safety complies with contractual agreements; with 29 CFR 1926, Safety and Health Regulations for Construction; and with various consensus standards.

Solicitation Input: EHS has drafted an extensive section describing subcontractor Safety Plan requirements. This section attached to solicitations will provide the bidder with the opportunity to understand the Safety Plan requirements before the project is awarded. In addition, EHS has provided a set of evaluation criteria that must be considered when reviewing proposals, including Experience Modification Rates, Days Away/Restricted or job transfer rates, Total Recoverable cases, and Corporate Safety Plans.

Construction Subcontractor Webpage: EHS has provided information to DMS to develop a webpage that further details the requirements of a safety plan and the expected safe work practices of construction subcontractors. It provides instructions and templates for completing a project-specific safety plan.

Construction Subcontractor Safety Orientation: EHS has developed an online orientation module that each subcontractor employee must take annually. The

orientation module details who we are at the NCI at Frederick and Frederick National Laboratory for Cancer Research (FNLCR), what we expect from construction subcontractors, and what construction subcontractors can expect in return. The safety orientation is designed to assist construction subcontractors in understanding hazards in the workplace and safety policies that are applicable while working at the NCI at Frederick and FNLCR. Over 400 individuals have taken the Construction Subcontractor Safety Orientation to date.

Project-Specific Safety Plans: EHS has defined that a Safety Plan consists of six standard elements. It has been further mandated that the construction subcontractor must provide a satisfactory Safety Plan prior to proceeding with scheduled work. Hundreds of Safety Plans have been provided and accepted to date.

Construction Subcontractor Monitoring: EHS plays a vital role with monitoring construction subcontractor work activities and handling construction subcontractor safety violations. EHS has made hundreds of construction site inspections to date, with violations noted in SIIMS and handled as appropriate.

Life Safety and Fire Prevention Program

The Life Safety and Fire Prevention Program (LSFP) ensures that appropriate building construction, fire protection, and fire prevention features and practices are maintained in order to minimize the danger to life and property from fire and related life safety hazards. Occupancy type, function, and other characteristics of buildings are considered throughout their design, operation, inspection, testing, and maintenance. The focus of this program is the protection of life, research materials, and property from loss due to fire and related hazards.

LSFP includes subject matter expert consultation; employee training; life safety and fire prevention inspections; and fire extinguisher inspection, testing, and maintenance. To ensure regulatory compliance and to mitigate multiple hazards, LSFP coordinates directly with the NIH Fire Marshall. LSFP also coordinates and consults with Fort Detrick Fire and Emergency Services (FD F&ES), as well as with all programs within EHS, building coordinators, animal facility managers, FME management and shops, and subcontractors specializing in fire system and extinguisher inspection, testing, and maintenance.

Advanced Technology Research Facility Environment, Health, and Safety

ATRF EHS is dedicated to reducing injuries, accidents, and environmental impact at the ATRF, and ensuring that activities at the ATRF are in compliance with all federal and state regulations, as well as with NIH guidelines and NCI at Frederick policies and procedures. EHS accomplishes this by providing quality training, comprehensive workplace evaluations, emergency response, and hazardous materials management from acquisition to disposal, and by managing regulatory information. At the ATRF, EHS encompasses all branches of safety within one office and performs tasks associated with biological, chemical, radiological, and fire and life safety, as well as with industrial hygiene and environmental and waste management services.

Industrial Hygiene (IH) services: One hundred ninety-five eyewash stations were tested per OSHA regulations. Work orders were submitted to FME for all eyewash stations requiring repairs, and follow-up was performed. Approximately 45 eyewash units were reassigned proper identification numbers to update the FME database inventory.

ATRF EHS researched and updated individual chemical, biological, radiological, and equipment inventory lists. As a result, new or updated signage was posted in 10 laboratories to fulfill regulatory requirements as well as alert lab users, visitors, and emergency responders to specific hazards within these laboratories. Additionally, the state of Maryland requires a list of all non-laboratory chemicals by facility. ATRF EHS reviewed and updated the list of approximately 147 non-laboratory chemical products and their accompanying safety data sheets (SDS).

During the course of the year, 22 safety shoe/eyewear forms were signed, 9 fall protection plans were created and approved for FME, 3 SDS were researched, reviewed, and approved for the Biopharmaceutical Development Program (BDP), and 5 Ergonomic Worksite Evaluations, 1 consultation, and 10 follow-ups were performed to improve employees' worksites with the intent of reducing occupational ergonomic-related injuries. ATRF EHS assisted with one FME confined space entry.

Also, ATRF EHS helped FME schedule all chemical fume hoods for certification.

Two incidents were reported at the ATRF, resulting in investigations and corrective actions and completed reports. Eighty-five reported deficiencies were noted at the ATRF, and nearly all were corrected and closed out within 45 days.

ATRF EHS also provided refresher training on waste and general safety for FME custodial workers, general safety refresher training for BDP staff, and refresher waste training sessions for various lab staff.

Environmental and Waste Management Services: ATRF EHS performed weekly hazardous waste inspections for all ATRF waste areas throughout the year, and there have been three hazardous chemical waste and eight biohazardous, or medical waste, pickups. ATRF EHS shares waste disposal tasks with the EHS Waste Department and provided numerous waste container drop-offs and autoclave waste transports throughout the year.

An inspector from the City of Frederick's Wastewater Pretreatment Office toured the ATRF to review effluent records, spill response plans, and supplies, and to ensure pH neutralization and steam sterilization equipment is maintained per the facility's Wastewater Discharge Permit requirements. No violations were noted.

Two annual wastewater sampling reports and all required accompanying paperwork were submitted to the City of Frederick per our Significant Industrial User Wastewater Discharge Permit. The June sampling results showed above permit concentrations of three parameters, which resulted in two Notice of Violation letters to be received from the City of Frederick. EHS resampled, investigated facility activities to determine probable sources, and ultimately determined that the June results appear to be an outlier potentially related to non-representative samples. Results of the investigation were provided to the City of Frederick and NCI.

Biological Safety Tasks: During the contract year, ATRF EHS received and processed one DEA-controlled substance shipment and performed four DEA-controlled substance audits. ATRF EHS helped distribute Tax-Free Alcohol logbooks to authorized laboratory programs and performed 41 tax-free alcohol audits. ATRF EHS helped the FME hood crew with biological safety cabinet (BSC) certification scheduling for all BSCs at the facility.

Fire and Life Safety: The RAS Initiative Symposium brought approximately 500 guests to the ATRF. Fire safety guidelines were set in place for the symposium committee, and assistance was provided by the ATRF safety officer for all safety-related requests prior to and during the symposium. ATRF EHS had its annual fire evacuation drill in October with assistance from EHS personnel from the main campus, FME, and security. Forty-five hot work permits were approved by the ATRF safety officer during the contract year.

Radiation Safety Tasks: The ATRF has three radioisotope programs, one electron microscope program, and one new X-ray program. ATRF EHS assisted the Radiation Safety Office in providing radiation safety support to 60 individuals who are approved to manipulate radioactive materials and/or work with electron microscopes at the ATRF.

ATRF EHS performed 100 percent of regulatory-driven contamination surveys each month. On average, 45 samples were taken each month at the ATRF (with no contaminated sites above the MDE-driven action level of 220 dpm). This low overall level of radioactive contamination demonstrates that facility radiation workers are keeping exposures to radioactive materials as low as reasonably achievable (ALARA). ATRF EHS staff also performed a six-month inventory for all ATRF radiation programs and a physical inventory for all radioisotopes present, as well as a uranium inventory for the electron microscope program. The ATRF EHS processed three incoming radioactive materials shipments within three hours of receipt, in accordance with internal policy and the Code of Maryland Regulations.

Facility Renovation Projects and Construction Services: ATRF EHS provided assistance to FME for projects and special assists by reviewing and approving safety plans; providing room safety clearances and completion walkthroughs; and issuing hot work permits and assisting with facility alerts and outages.

ATRF EHS assisted with determining flammable loading limits for all CCR labs temporarily moving into the facility and has provided much needed facility related safety information to the CCR staff prior to their move dates. Move meetings are being attended, and pre-occupancy and post-occupancy walkthroughs have been performed for swing moves currently in order. ATRF EHS has provided facility safety documents and information for the new ATRF website.

Vaccine Clinical Materials Program Environment, Health, and Safety

Vaccine Clinical Materials Program (VCMP) EHS provides the full-range of safety support functions to the Vaccine Research Center's Pilot Plant facility. This includes hazardous waste management, fire and life safety, regulatory compliance at the state and federal level, industrial hygiene, biological safety, and environmental management. These functions are balanced within both NIH and NCI at Frederick guidelines and policies as well the FDA's current good manufacturing practices (cGMP) requirements for clinical vaccine production. EHS duties at the VCMP are carried out with one safety officer providing onsite support, but utilize a coordinated effort with VCMP FME and NCI at Frederick EHS staff to ensure safe and successful operations.

Environmental and Waste Management Services: As a manufacturing facility, the VCMP at times produces both bulk chemical and biological wastes. The facility currently uses a batch steam-sterilization system for liquid biological waste processing. The VCMP also uses a pH neutralization system to process many chemical items in an effort to minimize waste disposal requirements and costs.

VCMP EHS works with EHS Waste Management in transport, packaging, consolidation, and processing of hazardous materials/waste produced in both laboratory and production activities. VCMP EHS also works with EHS Waste Management in the annual Emergency Planning and Community Right-to-Know Act (EPCRA) Tier 2 inventory and reporting of large-volume hazardous materials storage and usage. For VCMP waste disposal, there have been four chemical waste shipments and weekly pick-ups of regulated medical waste.

Fire and Life Safety: The VCMP conducted an annual evacuation drill on October 15, 2015 with assistance from EHS, FME, and Protective Services staff. Annual fire extinguisher training was conducted onsite in April of this year. VCMP Protective Services staff also provides regularly scheduled CPR/AED training open to all VCMP staff. Sixty-four hot work permits were approved and submitted to EHS since September of last year.

Industrial Hygiene Services: An annual performance test was performed on all 30 VCMP eyewash stations, per OSHA regulations. All noted deficiencies were quickly corrected by VCMP FME staff.

The VCMP produces vaccine materials for Phase 1 and Phase 2 clinical trials. To comply with both FDA and OSHA regulations, safety data sheets (SDS) are authored for all of these materials. VCMP EHS works with VCMP Quality Assurance to ensure compliance with the Globally Harmonized System for Hazard Communication on these SDS documents. VCMP EHS also manages the SDS inventory for the VCMP's 554 chemical items/products.

Over the past year, VCMP has successfully completed a confined space entry into its 2,000-liter bioreactor for the purposes of repair and maintenance. In July of this year, the VCMP corrected its most long-standing deficiency by installing a refrigerant monitoring system in its Chiller Building.

In the past year, there have been 12 incidents reported at the VCMP resulting in an investigation. The deficiencies found in 11 of these have been noted, resulting in both procedural and engineering control changes at the VCMP.

Facility Renovation and Construction Projects: VCMP EHS provided assistance to FME in review of safety plans and hazard assessments for various renovation and construction projects. These projects include:

- Installation of a refrigerant monitoring system
- Renovation and establishment of a new GMP warehouse at 7114 Geoffrey Way
- Conversion of a laboratory space into a walk-in refrigeration unit
- Upgrades to all VCMP uninterrupted power supply (UPS) units
- Installation of a continuous-flow biowaste treatment system

EHS was involved in every step of these projects, from design to substantial completion and into operation. EHS coordination and cooperation with FME for all upgrade and renovation products led to safe and uninterrupted operations at the VCMP. As a result, the VCMP completed production of Phase 1 clinical materials for use in Zika vaccine trials this past July.

Biological Safety: VCMP EHS provides ongoing support to laboratory, facilities, and manufacturing staff in all areas of biosafety. New tax-free alcohol permits were obtained for the VCMP's new warehouse at 7114 Geoffrey Way.

Protective Services

Protective Services Access Control performed more than 7,000 identification badge and cardkey transactions. The Protective Services Homeland Security Presidential Directive 12 (HSPD-12) Program Office fingerprinted and/or processed more than 1,600 FNLCR employees for their personal identity verification (PIV) badges.

EHS is part of a project team to upgrade the Pegasys P2000 cardkey system across NCI at Frederick and off-site facilities. This project team includes stakeholders from FME, IT Security, and DMS, and coordinates with the NIH HSPD-12 Program Office.

Protective Services continues to provide daily inspections of NCI at Frederick fleet vehicles. Such inspections include refueling these vehicles daily and making certain vehicles are washed as needed. Passenger shuttle service to NIH was maintained throughout the year, and more than 6,500 passengers used the service. In addition, Protective Services continues to run "special shuttles" to provide guest researchers transportation between their Frederick hotels and Fort Detrick, and transportation to the NIH campus for seminars. Protective Services continues operating an after-hours shuttle service from Building 426 to points outside the Fort Detrick gates that close early, for employees who walk to work and are affected by the new gate closing times. "Special shuttles" must be approved by the EHS director.

Protective Services closely monitors scientific equipment located at NCI at Frederick, the ATRF, and the VCMP Pilot Plant. Protective Services officers performed over 200,000 foot patrols at these

locations. These patrols have resulted in the discovery of more than 15,000 scientific and utility alarms, more than 2,300 security violations, and more than 150 fire safety violations (such as portable heaters or coffee pots left on).

Protective Services continues to monitor and grant access to irradiator room users, and during the past year, more than 600 FNLCR employees were granted access.

Protective Services dispatch officers continue to greet and register NCI at Frederick visitors. Over the past year, more than 3,700 visitor badges were issued.

Protective Services officers continue to perform monthly checks of fire extinguishers, AEDs, exit lights, emergency lights, and exterior lights. During this period, more than 500 work orders were submitted to have faulty lights repaired.

Occupational Health Services

The Occupational Health Service (OHS) program provides comprehensive occupational health services for all employees at NCI at Frederick. The mission of OHS is to maintain and enhance employee safety and productivity; comply with federal and state regulations; and decrease costs associated with absenteeism, disability, workers' compensation, and health insurance, by keeping NCI at Frederick employees aware of potential health risks. The multifaceted OHS program focuses on disease and accident prevention, treatment, management, and rehabilitation, which are accomplished through comprehensive screening and surveillance programs, health promotion, and one-on-one employee consultations.

OHS works closely with the local community health resources, including our medical consultant Dr. Anusha Belani (chief of Epidemiology for Frederick and specialist in infectious disease) as well as other EHS program staff. The OHS team has developed collaborative relationships with experts at the CDC, NIH, NIAID, and the local scientific communities as well as the local emergency medical services (EMS) team. Through these relationships, OHS works to ensure that NCI at Frederick employees receive the benefit of every work-health-related discipline in managing occupational medical concerns and issues specific to any/all potential or sustained occupational injuries and illnesses.

An essential element to performing our role is the development and maintenance of trust by NCI at Frederick employees in the care and confidentiality of services provided by OHS.

OHS has many varied medical surveillance programs in place. The type and number of programs continue to expand to meet the needs of our biomedical research community. Through its representation on the IBC and ACUC, OHS is able to modify existing protocols to meet accepted workforce health protection requirements and recommendations. New agents are discussed at the IBC and ACUC meetings, and medical protocols for the health and safety of employees are developed and approved.

OHS works closely with EHS to monitor the safety of all NCI at Frederick employees, both on campus and at our offsite facilities. This team approach helps with accident investigation and with the evaluation of the surveillance programs. Collaborating with EHS, which individually screens each employee for potential hazards in the workplace, allows OHS to offer appropriate medical surveillance programs and necessary vaccinations. OHS is diligent in teaching employees the Emergency 1-2-3 approach to first aid. OHS promotes awareness to ensure that employees know and understand all possible risks to themselves while in the workplace. In addition, when a workplace injury occurs, OHS offers immediate medical treatment and follow-up for prompt return to work.

OSHA reports, as well as accident investigations and corrective actions, are reviewed monthly by EHS and OHS to track the severity of occupational illness so that trends can be identified.

Work-related injuries/illnesses are treated in the Occupational Health Clinic by OHS clinicians. All workers' compensation claims are reviewed by an independent medical consultant and discussed with the provider. This in-house treatment enables OHS to help reduce employees' medical expenses and lost time, as well as workers' compensation costs. Most importantly, it enables a continuity of medical care that is the best practice for the health and welfare of NCI at Frederick employees. The OHS staff includes a certified worker's compensation nurse case manager and licensed staff who are knowledgeable in the unique risks and job requirements of working in a biomedical research facility. They work with employees, their supervisors, Human Resources, and, if required, outside medical providers to enable employees to return safely to work following injury or illness. In addition, OHS works very closely with our Worker's Compensation Insurance carrier to manage referrals and facilitate care to injured workers.

OHS works with NCI at Frederick staff in performing return-to-work exams for the management of complicated disability- and work-related cases, and serves as a valuable resource for other work-related issues. Episodic and acute services offered by OHS include: minor laceration repair, fracture stabilization, bumps, sore throats, ear infections, and other "just don't feel well enough to stay at work" conditions. This service encourages immediate and appropriate attention to urgent care health problems, which increase employee productivity by reducing the need for, or length of, sick leave and lost work time.

Urgent care is the treatment of a disease, illness, or injury when presented on an episodic basis. The disease, illness, or injury treated in an OHS setting is

usually acute, and with treatment, is fully corrected in seven to fourteen days. The services are provided five days each week, for an average of 60 minutes each day, from 10:30 a.m. to 11:30 a.m., and an appointment is not required. Nurse practitioners and nursing staff are providers in the OHS clinic. Because the focus is only on episodic problems, the OHS practitioners do not provide obstetric services, in-hospital admission, or long-term management of chronic diseases such as cancer, diabetes, heart disease, hypertension, or other conditions requiring long-term medical management.

OHS clinicians are on call 24 hours a day, 7 days a week, to respond to after-hours biological emergencies. OHS has streamlined support to the HIV production lab on campus and an ATRF lab working with frank oncogenes to establish a lock box with post-exposure medications. This lock box on site would necessitate a call to the OHS clinician on call to provide the combination and consultation for timely post-exposure medication dispensation.

Significant Achievements

Clinical Laboratory Improvement Amendment (CLIA): The OHS clinic was part of the 2 percent of federal sites selected to gather information about the Certificate of Waiver designation. The survey focused on evaluating the ability of the laboratory to ensure quality test results (glucose, urine dip, strep testing) based on applicable requirements. The survey was completed, and OHS was in compliance with the CLIA Certificate of Waiver program.

American Red Cross Blood Drives: OHS hosted four blood drives during the year, collecting 121 units and meeting our goal at every drive.

Hearing Conservation Program: There are 61 employees enrolled in the Hearing Conservation Program. OHS provides annual audiograms. Each employee is individually assessed and evaluated regarding their current hearing protection. There is a one-on-one education with a certified hearing conservationist to teach about hearing promotion and proper utilization of hearing protection. Each audiometry test is evaluated by a licensed audiologist and summarizes results in accordance with the OSHA standard.

Reproductive Health: OHS conducted 11 pregnancy interviews. This program is voluntary but highly recommended to protect maternal health and fetal development. The supervisor, employee, and nurse practitioner meet to identify potential reproductive hazards in the employee's work environment. OHS collaborates with the NIH Lactation Coordinator to educate the employees as well as support the Lactation Program.

Blood and Body Fluids, and Other Potentially Infectious Material (OPIM) Program: EHS enrolls employees into the (BBP, OPIM) medical surveillance. OHS contacts the employee to educate them about the risk of working with bloodborne pathogens and offers the Hepatitis B vaccine. After counseling, 169 employees started the Hepatitis B series.

Respiratory Protection Program: OHS performed respirator protection evaluations on 334 NCI at Frederick employees. These included all employees whose work scope requires them to wear a respirator and obtain appropriate fit testing. OHS is responsible for notification and completion of all OSHA-mandated respirator fit testing for employees at the main campus at NIH, NCI at Frederick, offsite locations (VCMP, ATRF) and provides pulmonary function test and fit testing.

Animal Care Worker Program: OHS provides a medical screening program for all NCI at Frederick employees who enter into an animal facility and/or work with live animals or tissue. This screening identifies those employees who may present with allergy signs and symptoms and aims to prevent occupation-related asthma. OHS collaborates with EHS in the allergy-prone animal worker to identify PPE and to evaluate work-site practices.

Offsite, nonhuman primate workers are offered special shuttle service to OHS semiannually for medical surveillance exams, thus ensuring improved AAALAC compliance and outcomes. Additionally, OHS has a staff member on the ACUC to provide enhanced support to the Laboratory Animal Sciences Program (LASP).

Advanced Technology Research Facility (ATRF) Services: OHS staffs a clinic with a licensed provider at the ATRF three days a week for four-hour periods without additional burden to the budgeted FTEs. The scope of services includes work-related visits, first aid for work-related injuries and illnesses, and minor urgent care. OHS reviews the ATRF Wellness Center physical activity readiness questionnaires and screens for those employees requiring additional clearance documentation from their private physician. OHS offers wellness events at the site including the Take a Hike events and blood pressure screening during clinic hours. OHS coordinated special training for research-related occupational medical safety including a lockbox for BSL2 lab with frank oncogenes and emergency treatment training for hydrofluoric acid. In addition, OHS piloted a trial of preplacement examinations at the ATRF clinic to assist the foreign nationals who experienced barriers entering the military base.

First Aid and Cardiopulmonary Resuscitation Program: OHS conducts first aid and CPR certification courses to employees at NCI at Frederick and offsite facilities. OHS coordinates with Protective Service officers to maintain current first aid kits and planned training for all employees who have extended or protracted working hours (such as those working at LASP and FME). Classes are offered every month.

Employee Assistance Program: The manager of OHS serves as a co-contracting officer's technical representative (COTR) for the Business Health Services (BHS; Employee Assistance Program) contract. Responsibilities include reviewing quarterly utilization and examining ways to communicate to employees about BHS services. A program was held for managers in coordination with human resources to raise awareness about EAP services.

Research Donor Program: OHS administers the NCI at Frederick Research Donor Program (RDP), which is an NIH Institutional Review Board (IRB)-approved research protocol. The RDP supplies anonymous donor blood and other human samples to researchers at NCI at Frederick. Utilization of the RDP has consistently increased, and investigators continue to express appreciation for these specialized services, as they are otherwise unavailable and are critical to the quality and continuity of their research.

These donations have helped the research for more 70 different programs. There are 264 donors in the program with 1,919 total donations for 90,421 milliliters of blood collected.

Medical Surveillance: OHS coordinates surveillance programs with EHS for employees whose job requirements may pose potential health risks. A Medical Surveillance Enrollment Form (MSEF), developed in conjunction with the Biological Safety Program, provides a complete, systematic method of collating medical hazard information specific to the employee's work functions. EHS is responsible for the distribution and collection of the MSEF.

Staff Development and Certifications: Clinical staff members participate annually in clinical nursing skills and are tested to prove clinical competency (e.g., administration of EKG, Morgan Lens, parenteral injections). All nursing staff members are certified in spirometry and hearing conservation. OHS has a certified RN workers' compensation case manager. The manager of OHS is certified in occupational health nursing and serves on the American Association of Occupational Health Nursing Board. OHS also has a representative on the Frederick County Chamber of Commerce Wellness Committee.

Wellness Program: The Wellness Program has been clearly branded and advertised as a separate program that is voluntary and is not included in the work-related annual exam offer. The Wellness Program offers influenza vaccines, cholesterol testing, blood pressure screening, the Take a Hike event, Healthy Heart Fair, and the Stepping for Stars campaign. OHS recognizes and supports a monthly wellness topic to educate the campus on health promotion and cancer prevention.

In efforts to educate all employees about health promotion and current research trends, OHS collaborated with Dr. Ligia Pinto to sponsor a summer seminar on human papilloma virus (HPV). This involved a screening of a movie followed by a panel of subject matter experts from the Frederick community including the Health Department, a local pediatrician office immunization coordinator, and the Department of Health and Mental Hygiene representing the HPV vaccination efforts.

International Travel Consultation: OHS provides international travel consultation to support the Ebola vaccine clinic. The need for this service was dependent on the establishment of vaccine center trials. OHS provided 48 travel consults including travel vaccinations and medication for travel specific health conditions.

Werner H. Kirsten Student Interns: OHS has supported and contributed to the WHK program by serving on the committee to upgrade the computer application process. In addition, OHS has served as a clinical site to host two students. OHS provides screening and training for all students in the WHK program and has implemented changes to protect their health information.

Cancer Research Training Award (CRTA): OHS supports the CRTA student program providing medical screening and surveillance. In addition, OHS is privileged to have several CRTA students in the clinic interested in a career path in medicine.

Statistical Overview: OHS logged 6,629 encounters during the period from September 1, 2015 through July 26, 2016. The ATRF satellite clinic logged 526 encounters.

FACILITIES MAINTENANCE AND ENGINEERING

During this contract year, the Facilities Maintenance and Engineering Directorate (FME) successfully completed a wide array of projects for NCI at Frederick. The following highlights some of these accomplishments.

Repave Service Lanes, APA. The purpose of this project was to remove deteriorated road surface and sub-surface and to re-pave the remaining two service lanes in the APA area. The existing service lanes were in poor condition, with numerous areas of missing pavement due to the aging of the surfaces. These conditions created potential trip hazards and marked difficulty for workers moving hand carts to transport animal cages between Building 1021 and the outlying APA buildings. Phasing of the construction was a major consideration as the contractor was required to maintain access at all times for both pedestrians and rolling carts to Laboratory Animal Sciences Program (LASP) Buildings 1027 and 1029. This project succeeded in providing a safe surface environment for daily work requirements, improved drainage, and visually upgraded the area.

Install Emergency Generator, Building 321/322. This project was required to provide emergency power backup to scientific equipment containing Division of Cancer Treatment and Diagnosis (DCTD)/Developmental Therapeutics Program (DTP)/Biological Resources Branch (BRB) Repository clinical agents valued in excess of $1 million. The investment in these materials must be protected prior to distribution to clinical centers. DCTD's preparation of culture systems from patient-derived surgical samples is also undertaken in Building 321. These are prepared from patient biopsy material that is critical to the program. Loss of any patient material must be guarded against, as additional samples are unattainable in most cases. All building automation system (BAS) panels, network equipment, scientific alarm panels, and the card key system have been connected to the new standby generator.

Replace Chiller #1, Building 571. This project entailed the replacement of the existing 514-ton chiller with a new 530-ton chiller. The existing chiller utilized R-11 refrigerant, which is an ozone-depleting substance regulated by the United States Environmental Protection Agency (USEPA). This project is part of NCI's ongoing effort to phase out the use of these refrigerants on campus. This project provided a more efficient and environmentally friendly system by upgrading the chiller and eliminating obsolete equipment.

Add Generator Fuel Tank, Building 1066. This project added a 480-gallon double-wall diesel/fuel oil auxiliary fuel tank to the existing Building 1066 emergency generator. The freezer repository in Building 1066 contains many valuable scientific samples that must be maintained in the event of a power outage. Adding the auxiliary fuel tank increases the generator run time during an extended outage at full load from 31 hours to 58 hours, providing an additional margin of safety for the repository.

Renovate for Robotic System, Building 432. This project renovated Room 129 and relocated a 30 kW generator to accommodate the installation of a new robotics platform that allows automated high-throughput and/or high-content image analysis of cells. The selected robotic platform required access to an appropriate electrical supply as well as a backup generator to allow the system to run around the clock without interruption. A camera system allows an operator to view the process remotely. Remote access to the system allows corrections to be made in a process when the lab is not occupied. Design for this complex renovation was performed in-house by the FME design team. Extensive coordination with the program and the equipment supplier was necessary to ensure functional interface between the new scientific equipment and the site-specific building utilities.

Install Rees Wireless Alarm Systems, Buildings 535, 538, 560, 562, and 469. The purpose of this project was to upgrade the subject buildings to Rees wireless alarm systems in order to improve alarm service and reliability. The project included the installation of sensors on all freezers, refrigerators, and incubators, and provided Rees central work stations in respective buildings for connection to the central server in Building 362. All outdated scientific alarm panels, equipment, and wiring have been removed. This work was accomplished with minimal disruption to scientific activities.

Most important accomplishment for the reporting period:

FME has been exceptionally responsive to the dynamic requirements of the refurbishment initiative. Considerable effort has been expended in optimizing staffing levels for all departments in relation to projected workload and NCI expectations. At the same time, FME has worked closely with Leidos Biomed's Contracts and Acquisitions Directorate (C&A) to secure additional architectural and engineering (A&E) providers and acquire temporary engineering and construction talent from outside providers. The Human Resources group in Leidos Biomed has been instrumental in supporting FME's need for the recruitment of additional staff for this effort. The team has worked closely with NCI leaders and scientific stakeholders and effectively adapted to continuous change in the needs of the NCI. The FME team has developed aggressive approaches and schedules that satisfy the NCI's interest for the FY2016 refurbishment efforts. Major efforts are underway as the refurbishment projects move forward. FME has successfully launched over 40 work orders, which are proceeding on schedule in support of the refurbishment initiative.

Information Technology Operations Group

The IT Operations Group (ITOG) is a part of the DSITP within Leidos Biomed. ITOG is responsible for computational servers, storage servers, the virtual machine infrastructure, and the FNLCR network. ITOG focuses on implementing enterprise IT best practices in the areas of computational services, storage, backup, and archiving; batch and application support; server consolidation and virtualization; network infrastructure; unification of voice, teleconferencing, and video communication technologies; and improved infrastructure for collocation of dedicated servers.

Architecture

The architecture group participated in the effort to develop a long-range strategic plan to address the future research needs of both FNL and CBITT. There were 12 groups contributing to the effort, with ITOG directly involved in seven of the groups, including High-Performance Computing, Storage, Databases, Networking, Virtualization, the Cloud, and Unified Communications. The 12 groups developed long-range plans to provide the basis for FY2017 IT infrastructure budgets.

Storage and Server Operations

- ITOG successfully concluded a three-year project to back up TCGA data for CGHub at the University of California at Santa Cruz. The storage eventually reached 2.6 petabytes (PB) of storage and 124,000 files. The cost of the project was reduced from an original estimate of $700,000 to $182,000 by using open-source products and backing up to an existing tape library.
- The use of an open-source database has dramatically increased over the last several years to the point where we now have over 400 databases, with over 50 databases added in the last year. The popularity of these open-source relational databases is primarily due to lower costs and ease of use over traditional relational databases like Oracle. To keep up with this heavy demand, ITOG has implemented a new quad node cluster in a shared mode to support the latest version of MariaDB, the follow-on open-source version of MySQL. This facility significantly enhances our ability to support new and existing instances of open source relational databases.
- The production and development Oracle servers have recently been replaced with host platforms that offer a substantial boost in input/output (i/o) performance. In particular, this included dual solid-state drives (SSDs), a Flash buffer cache, high-performance disk controllers, and disk storage expansion. The previous host platforms had reached saturation points with the disk controllers, which were impacting the system's ability to meet demand. The new host systems are providing excellent performance results for the growing Oracle operations and instances.
- The Cleversafe object storage facility has been deployed to four geographically dispersed locations: Building 430, ATRF, CIT-Bethesda, and CBIIT Shady Grove. The system is currently offering 1.2 PB of storage across four clusters. The facility is intended to support research data that is inactive yet must remain available. Data protection is provided through the erasure coding of the data that is spread across the four clusters, allowing data recoverability in the event of a disk(s) or single site failure.
- The virtualization operations have been greatly enhanced by the addition of two Tintri T880 appliances, which provide a flash and disk array that is virtual machine (VM)-aware. It is simply presented as a storage resource to the virtualization administrator. This has removed the additional complexity that was taxing the storage administrator's team, as they had to constantly make storage area network (SAN)-based storage available for each VM. As the number of active VMs approached 800, the storage complexities increased. The new appliances have drastically simplified the storage allocation for VMs, allowing quick turnaround for the customer requests.
- ITOG staff rebuilt the Cactus web applications to allow it to run in the Frederick Demilitarized Zone (DMZ). This allowed the system to meet the requirement for all outwardly facing systems to be placed in a DMZ. The rebuild required the updating of web applications and databases into currently supported versions and the removal of hardcoded domain name system (DNS) names and Internet Protocol (IP) addresses from the applications and scripts. The new system was put into production in December 2015 with no major disruptions in service.
- ITOG supported Bioengineering Research Grant (BRG) VMs with the upgrade of 40 VMs from Ubuntu 12.04 or CentOS to Ubuntu 14.04, cleaned up and standardized system configuration, implemented IPv6, and addressed numerous security issues. Completed 30 software builds and deployment processes in Jenkins CI, including high-profile production web applications such as Biospecimen Research Database (BRD) and Comprehensive Data Resource (CDR) application. Eighteen new VMs were completed for new projects.

Network and Telephone Group

- Substantial completion of the Fort Detrick Voice over Internet Protocol (VoIP) Project. There were 4,210 end points placed, including 2,732 phones and 721 modems and faxes. The new system's benefits include voice dialing, retrieving of voice mail through outlook, direct dial to Shady Grove and, when the rest of NCI goes to VoIP, call to NCI Bethesda. As part of the deployment, the Network and Telecommunications Group (NTG) deployed a dedicated network at the Industry Lane site for VoIP and the card reader system. The network allows employees at Industry Lane to

participate in the larger Fort Detrick VoIP system in addition to providing network connectivity for the card reader system.

- ITOG, with the help of Data Management Services (DMS), completed the implementation of IPv6 across campus to all servers and desktops that could allow IPv6. There were 4,407 endpoints enabled including 595 servers and 3,812 workstations and laptops.
- The Network Access Control (NAC) Project had completed testing phase including the implementation of three buildings and was ready for full implementation. NIH has since cancelled any further development with the Extreme NAC solution.
- Building renovations. NCI has plans to completely renovate six large buildings on the NCI at Frederick Fort Detrick campus over the next two years. NTG is involved with planning network local area network (LAN) closets, network connectivity, and assisting with moving users to temporary locations as buildings are renovated.

INFORMATION SECURITY AND COMPLIANCE OFFICE

The Information Security and Compliance Office (ISCO) within DSITP is the official NCI at Frederick office for the local information systems security officers (ISSOs), and it serves as the point of contact for NIH security compliance requirements and responses to security incidents. ISCO is responsible for security assessments, waivers, IT risk assessment, and security incident handling, and for working with NCI at Frederick IT groups to integrate best practices into IT planning and implementation. ISCO is staffed with one assessor (and is in the process of hiring two others), two alt. ISSOs, and two risk experts. ISCO provides security guidance for the NCI at Frederick campus, which has approximately 8,000 IP addresses with 2,500 users.

For 2015–16, ISCO has successfully completed several initiatives within NCI at Frederick. Listed below are several accomplishments within the ISCO:

Security Assessments

ISCO conducts Federal Information Security Management Act (FISMA)–related security assessments each year. In addition to assessing new systems, ISCO conducts annual assessments each year to confirm authorized systems are up-to-date on security requirements. The following systems received new authorizations to operate, which requires a full review of all National Institute of Standards and Technology (NIST) 800-53 controls:

- Genomic Data Commons (GDC)—provides cancer genomics data in a co-localized database to serve as a foundation for future expanded data access, computational capabilities, and bioinformatics cloud research. The GDC will be a core component of the National Cancer Moonshot and the president's Precision Medicine Initiative (PMI). The system required reaccreditation after moving into production and adding an Amazon cloud component.
- The Comprehensive Biospecimen Research (CBR) system is an information system located at the Van Andel Research Institute (VARI) that is designed to enforce standardized collection, processing, storage, distribution, and analysis of biospecimens, including their derivatives and associated data.
- The Bionimbus Protected Data Cloud is a secure self-service portal that enables researchers to set up their own VMs to access the data in Bionimbus, which currently includes selected data from TCGA.

Annual Vulnerability Assessment

The NIH Vulnerability Assessment team performs annual testing on our network to identify any system deficiencies. Due to the efforts of the ISCO over the last fiscal year, the assessment team struggled to find any major vulnerabilities. The Vulnerability Assessment team had to work harder to provide results. This increase in the depth allowed NCIF's security posture to increase compared to previous years. ISCO was able to work with system administrators to remediate all findings before the required NIH completion date.

Improved Intrusion Detection Monitoring

ISCO has improved our intrusion detection system (IDS) by increasing the depth of the tools and increasing capabilities. We are now able to capture and analyze network traffic and link the information to tailored alerts. These additional capabilities allow the ISCO to verify alerts by inspecting the actual traffic, greatly decreasing the time to action if warranted. The additional data will not only help ISCO detect actions that attempt to compromise the confidentiality, integrity, or availability of IT resources for all of NCI at Frederick but can also provide attribution. The additional depth and breadth of the IDS security tool, paired with our security information and event management (SIEM) application, Splunk, has significantly increased our overall security posture.

CONTRACTS AND ACQUISITIONS

The Contracts and Acquisitions Directorate (C&A) was reorganized during FY2015 as part of the organizational restructuring of Leidos Biomed's Frederick National Laboratory for Cancer Research (FNLCR) operations. This restructuring received NCI approval in July 2015.

Responsibility for all FNLCR prime contract administrative functions was assigned to C&A in January 2015. The consolidation of all prime contract responsibilities within C&A facilitated the joint efforts of NCI and Leidos Biomed to establish a new Indefinite Delivery/Indefinite Quantity (IDIQ) task order contract during FY2015 that works in tandem with the Operations and Technical Support (OTS) contract. C&A continues to be responsible for purchasing, research and construction subcontracts, intellectual property, and logistics.

Robert Mason was appointed as C&A directorate head in April 2015. He has over 36 years of government and commercial contracting experience and has been with Science Applications International Corporation (SAIC)/Leidos Biomed for over 21 years, during which time he has held positions of increasing responsibility in federal, commercial, and international contracting. In particular, he has extensive experience in working with IDIQ task order contracts. This experience was instrumental in the development, negotiation, and implementation of the new IDIQ task order contract that was agreed to by NCI and Leidos Biomed in September 2015.

Acquisitions

The Acquisitions Department (consisting of purchasing, research subcontracts, construction and facilities services, and procurement compliance) made significant transformational changes to focus on service delivery, efficiency, effectiveness, transparency, and accountability. Various measurements, metrics, and performance metrics were established, including cycle time, competition, and supplier diversity. Process enhancement initiatives have resulted in work elimination, simplification, controls, and quality improvement in subcontractor invoice review and processing. Other notable process changes included: a standardized risk assessment evaluation methodology, a revised consulting engagement procedure, and a means for validation and collection of cost savings. Subcontracts personnel program assignments were realigned to provide team support within departments. Spend analytics were performed to focus on commodity categories in Purchasing to establish cost savings goals and strategic sourcing initiatives. We also updated the functional compliance self-governance program for procurement. Activity among the respective portions of the Acquisitions function is as shown:

Acquisition Spend Sept. 26, 2015 – YTD Through July 15, 2016

Department	Spend	# of Actions
Purchasing	$130,698,445	44,454
Construction & Facilities Services	$19,918,480	296
Research	$110,175,481	369
Total	**$260,792,406**	**45,119**

Total commitments (spend dollars) grew 14 percent for the Acquisitions function for a comparable period in FY2015; total transactions grew 5 percent, although construction and facilities service and research subcontracts grew 48 percent and 52 percent, respectively.

Steve Grumbach was appointed Director of Acquisitions in October 2015. Mr. Grumbach is an experienced senior leader in strategic sourcing, subcontracts, procurement, and supplier management with more than 25 years in senior procurement leadership positions at SAIC, IBM, KBR, Battelle, and DynCorp.

Purchasing

The Purchasing Department is responsible for the acquisition of commercial goods and services, including equipment maintenance and fleet services, as well as management and training for program areas using the blanket order and credit card programs.

Outreach Initiatives

- Purchasing has conducted two refresher courses on COR360 and the CostPoint Credit Card program. There are two more scheduled in July and August. These courses are open to all authorized administrative support personnel to train on efficiency and accuracy in the procurement process in support of the NCI and Advanced Technology Research Facility (ATRF) labs.
- Purchasing will be conducting Capital Equipment Purchase Request Training in July and August.
- Purchasing is hosting a Small Business Vendor Show in September. A 20% discount on lunch for attendees during the event has been negotiated with the Discovery Café.
- Purchasing User's Committee: The Purchasing User's Committee continues to work to improve the procurement and program area communication process and strive for the most efficient system possible. The committee provides a dialog between program areas and C&A to discuss ideas and enhance communications to ensure an effective and compliant procurement process meeting the needs of both entities.
- Purchasing was part of the SMART PROC Small Business Conference: Connecting Government, Industry, and Academia.

Strategic Sourcing and Commodity Management Initiative

Purchasing developed a new initiative in January 2016 to evaluate commodities using spend analysis. The spend analytic gave us the ability to focus on our top commodities and implement a commodity management and strategic sourcing program. The top commodities represent about 88 percent of our total spend. Each top commodity has a commodity owner responsible for initiating action plans based on findings. The top commodities are animals and animal supplies, capital equipment, industrial supplies, lab supplies and biologicals, commercial research services, and service maintenance.

Commodity Management Accomplishments

- A Master Agreement with GE Healthcare has been established and includes free freight on all standard orders, including capital equipment. This is a savings of $16,000 for the first and second quarters.
- Negotiated a waiver of all freight charges from a laboratory equipment provider, Government Scientific Source, thereby avoiding more than $12,500 in estimated freight costs for 2016.
- The top four Commercial Research Services vendors provided a savings of $238,000 in the first and second quarters.
- Negotiated prompt delivery (24 hours) at no additional charge for 90 percent of lab supply stock.
- Cost savings on purchase orders (one-time tactical procurements, not credit cards or blanket releases) is $1.2 million so far for this contract year (2.26 percent of total spend).
- Second quarter cost savings of $441,865 on capital equipment, 6 percent of total spend.

Metrics

Purchasing is continually searching for new small business suppliers. This has expanded the competitive market. Fifty-four new small business vendors registered on the Leidos Biomed Potential Vendor Database, including ten woman-owned, six small disadvantaged, ten service-disabled veteran-owned small businesses (SDVOSBs), three historically underutilized business zone (HUBZone), and ten 8(a) businesses.

Purchasing continues to provide acquisition support at or near target performance milestones. Accordingly, some important measures of requisition actions are: 96 percent of material and supply requests were processed within 10 calendar days (target is greater than 90 percent) and 94 percent of radioisotope requests were processed within the established same-working-day metric (target is greater than 90 percent; this result reflects purchase requests (PRs) that were submitted late, as well as those that were provided without the requisite Environment, Health, and Safety [EHS] signatures, both of which cause processing delays).

Of the capital equipment requests processed, 83 percent of requests between $5,000 and $150,000 were processed within the established 25-calendar day metric (target is greater than 85 percent). There was marked improvement over the first three quarters of the year: first quarter was 62 percent; second quarter, 88 percent; and third quarter, 100 percent. The improvement in the performance levels over the last three quarters is due in large part to the addition of personnel in the Equipment and Maintenance Purchasing Team. Capital equipment requests over $150,000 have also shown improvement in the third quarter, with 83 percent within the established 30-calendar day metric (target is greater than 85 percent) versus the same timeframe last year, when 60 percent were within the established 30-day goal.

Construction Subcontracts and Facilities Services

The Construction Services Department (CSD) is responsible for all architecture and engineering, construction, and other facilities service requirements, including the lease and utilities of off-site locations supporting NCI at Frederick operations.

During the year, CSD's primary focus has been supporting the refurbishment projects. To that end, CSD has developed and executed multiple refurbishment project initiatives. The two major initiatives that have expedited refurbishment projects schedules are: (1) Developed an expedited procurement plan that fast-tracked the onboarding of additional engineering services needed for the refurbishment projects. This expedited procurement involved the evaluation, negotiations, and audit process that resulted in a contract award within 21 calendar days from initial direction to award. (2) Modified the workflow approval process with the NCI to allow advance authorization of architect and engineer services to begin work up to a 35 percent design.

In addition, CSD worked with FME to expedite the development of a statement of work (SOW) and award of a snow removal contract this winter, making the award within 46 calendar days from initial direction. The SOW, along with the type of agreement, was proven beneficial, as we received a blizzard of over 30 inches in late January shortly after the execution of the agreement that resulted in very favorable performance by the services supplier.

Research Subcontracts

Research Subcontracts (RS) is primarily responsible for the subcontracting of research-related work and various other services not commercially available in order to support the

research activities of FNLCR, NCI, and other NCI and NIH programs. RS is currently managing approximately 520 subcontracts, totaling approximately $297 million. Many subcontracts require specialized acquisition strategy, and many are in support of major national research initiatives. Some of the noteworthy accomplishments and initiatives are noted, as follows:

PREVAIL Clinical Trials: RS provided continued subcontract support to the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) clinical trials in West Africa. The focus of support during this period shifted from emergency operations to sustainment. This shift in focus has resulted in cost-efficient activities such as, but not limited to, closing out emergency agreements that had been in place and taking advantage of cost savings through sustained vendor relationships. Additional substudies at sites in Liberia, Sierra Leone, and Guinea are supported with domestic and international subcontractors and consultants as well.

FNLCR Repository: RS began taking steps to conduct an early recompete of the FNLCR repository subcontract. This decision was made based on multiple issues relating to technical and cost performance, as indicated in subcontractor award fee reports during the year. The incumbent repository subcontractor did not respond to the solicitation. Anticipated award to the new subcontractor will be July 29, 2016, with a 60-day transition period.

LDER: RS supported the Laboratory-Directed Exploratory Research (LDER) fund through subcontracts with educational and nonprofit research institutions to enhance innovation, creativity, originality, and quality of research activities while facilitating collaboration within FNLCR. Subcontracts were put in place for modeling cell heterogeneity dynamics in tumors in response to drugs and oral-immune profiles in human papillomavirus (HPV)-related oral cancers.

Cancer Moonshot Blue Ribbon Panel: RS supported the president's Cancer Moonshot Blue Ribbon Panel through the engagement of consultants focused on making the most of federal investments, targeted incentives, private sector efforts from industry and philanthropy, patient engagement initiatives, and other mechanisms to support cancer research and enable progress in treatment and care. Five consultants are engaged through RS in support of this presidential initiative.

VRC: RS is supporting the development of a clinical research study for an efficacious vaccine to prevent Zika virus infection through subcontracts with clinical research organizations conducting management and oversight of the studies at clinical sites throughout the Caribbean. In support of this effort, RS released a request for proposal to six vendors who were targeted based on their expertise within the endemic regions of the Caribbean and South America, as well as their expertise in planning and executing clinical trials. This effort has the potential to generate multiple agreements with domestic and/or international subcontractors.

Therapeutics for Rare and Neglected Diseases: Therapeutics for Rare and Neglected Diseases (TRND) projects continued throughout the year, with the issuance of task orders and subcontracts to support advanced toxicology, pharmacology, and drug development initiatives in support of sickle cell disease (SCD) and other rare disorders. RS continued to work closely with both the National Center for Advancing Translational Sciences (NCATS) and collaborators on complex issues to ensure the protection of proprietary rights in the development process.

Genomic Characterization Centers: RS provided continued support to the Center for Cancer Genomics (CCG) through established subcontracts with Genomic Characterization Centers (GCCs) that provide high-resolution, systematic, and comprehensive (genome-wide) characterization of cancer-related genomic alterations. The GCCs are continuing characterizations on biomolecules from the same biospecimens for optimal analytical integration for data comparability to reach the goal of processing 5,300 samples within the next year.

Cycle Time Metrics

New-award cycle time went from 71 days in first quarter FY2016 to 21 days in third quarter FY2016; modification cycle time went from 22 days in first quarter FY2016 to 21 days in third quarter FY2016.

Contracts Department

The Contracts Department is responsible for the day-to-day administration of the OTS and IDIQ contracts and task orders issued under the IDIQ. Contract administration efforts include tracking hundreds of compliance points and deliverable requirements as well as the full lifecycle management of each task order and negotiation of changes to contractual terms and conditions. Additionally, the department manages the company's conflict of interest program, provides contract document control and maintenance, and tracks contract service–level agreements and metrics. The department serves as a resource to the Leidos Biomed programs on matters related to contract terms, requirements, and regulatory interpretation and implementation.

The Contracts Department, as part of an overall initiative within the C&A, implemented a series of actions to increase effectiveness, quantify activities, and make appropriate process improvements to increase the quality of support for Leidos Biomed and government customers. These include:

- Developing a single point of contact within the Department for the NCI Management Operations

Support Branch (MOSB) to contact when data or other information are requested of Leidos Biomed. This consolidation reduces the organizational touchpoints for MOSB and allows the department to capture data related to the requests to improve response time and, in some instances, quantify the expenditure of time and costs necessary to respond.

- Through the broader use of data capture and analysis, the department will utilize analytical methods to provide objective measures of activities and performance as the basis for improvements in quality and processes.
- The expansion of a harmonized set of department standard operating procedures to ensure accuracy, completeness, and reproducibility of many of the department's processes.
- Contributions to a company-wide training initiative focused on increasing the knowledge of contracts-related information and requirements appropriate for varying levels of Leidos Biomed employees, managers, and senior staff.

As an additional aspect of overall contract/task order administration and compliance efforts, the department plays a key role in the proposal, award, and administration of the 18 task orders in effect during the period. The department continues to be increasingly involved in the management of the company's risk management program, policy development, and new program initiatives, such as the Combatting Human Trafficking Program. This period also included the quadrennial conflict of interest training for approximately 430 Leidos Biomed employees, which was created, distributed, and managed by the department. This training and related financial and other disclosures help ensure Leidos Biomed's compliance with its contracts free of conflicts of interests.

Intellectual Property

C&A is responsible for managing all intellectual property (IP), technology transfer, partnership mechanisms, and oversight of IP as it relates to our subcontractors.

Invention Reporting: From September 26, 2015 through September 25, 2016, 20 inventions were reported, and of these inventions, 13 were sole inventions with only Leidos Biomed inventors. One employee invention was the result of the Contractor Cooperative Research and Development Agreement (cCRADA) with the University of California, San Franciso (UCSF). A Patent Cooperation Treaty (PCT) application has been filed, and Leidos Biomed is in the process of negotiating an interagency agreement (IAA) with UCSF for handling the marketing and licensing of the intellectual property.

Collaboration Agreements and Beta Test/ Evaluation Agreements: Leidos Biomed collaborated with researchers at various institutions, including the Massachusetts Institute of Technology (MIT), GlaxoSmithKline, The New York Academy of Sciences, and Intel.

Cooperative Research and Development Agreements/Technical Service Agreements: The IP team completed negotiation of four cCRADAs, two Materials Cooperative Research and Development Agreements (MCRADAs), and two Collaboration Agreements, with eight agreements currently pending. Additionally, 46 Technical Service Agreements (TSAs) were executed, anticipated to bring in approximately $793,000 in revenue.

Material Transfer Agreements/Confidentiality Agreements: The IP team negotiated 61 Material Transfer Agreements (MTAs), including those for RAS, during the period, with approximately 15 pending final negotiation and execution. In addition, the IP team negotiated 64 confidentiality agreements with various entities, and 14 are currently pending.

Licenses: Leidos Biomed negotiated three (InFLUenza Patient-Reported Outcome) Flu-PRO license agreements for rights to utilize the copyrighted Flu-PRO instrument for influenza studies and respiratory syncytial virus (RSV). This included The Micron Group, Janssen Global, and GlaxoSmithKline. The IP team also negotiated licenses with Lonza for the use of the GS Gene Expression System and with MIT for the use of mouse models that will be used in a technical service offered by the Center for Advanced Preclinical Research (CAPR).

Procurement Compliance

The Procurement Compliance Manager (PCM) ensures compliance with all applicable prime contract and regulatory requirements, as well as applicable company policies across all acquisition departments.

The PCM has updated the Self-Assessment Program and Review forms based on changes driven by the Federal Acquisition Regulation (FAR) or other regulations. The compliance program sought to capture the general categories of findings and revised the review form. Once sufficient data is available based on these data points, the compliance program will generate trend reports on compliance adherence, providing data for procurement staff and management upon which to base performance assessments, oversight, and the review of files or process improvements.

The PCM has created a program that includes the posting of guidance documents on the site to help procurement staff successfully navigate high-risk procurement activities and provide general information on many other procedural areas. In addition, the manager worked with subcontracts management on specific FAR or NIH regulatory

requirements to ensure compliance (including salary cap, cost accounting standards, cost, and pricing data). The compliance program continues to work with procurement supervisors and managers to keep them informed of compliance issues related to file documentation and process risks.

In addition to the appointment of a PCM, C&A has also recruited and brought on board a seasoned professional, Mr. Dennis Chaloux, who has over 30 years of contracts and procurement expertise. Chaloux's experience includes the leadership roles for training and compliance for a Fortune 500 corporation, thereby bringing a new level of leadership in these areas to the C&A team.

Logistics Support and Property Compliance

The Logistics Support and Property Compliance Department provides receiving, distribution, warehousing, property accountability, mail, shipping, and transportation activities for FNLCR operations and related organizations.

Property Section: The C&A Government Property Section currently manages 35,293 items valued at $393,043,038. The section received and processed 3,261 items of accountable property valued in excess of $30.6 million into the property system. The Property Department is maintaining management of 1,652 items at various off-site subcontract locations valued at $19,818,435. As part of the responsibility under the contract to effectively manage surplus property and equipment, the Property Department has transferred 256 items of accountable property valued at $2,226,818 to other federal agencies and arranged for the donation of 28 items valued at $68,279 to educational institutions. In addition, the Property Department successfully reissued 78 items of accountable property valued at $360,581.64 from surplus to programs throughout NCI at Frederick.

Consolidated Warehouse Section: The C&A Consolidated Warehouse section maintains both a Central Supply and Maintenance Supply to enable the quick-reaction provisioning of materials and supplies to NCI at Frederick programs. Distribution of items from the Supply Warehouse is conducted via an online ordering system.

During this period, the **Supply Warehouse** received 12,249 requisitions containing over 28,869 lines for materials and supplies valued at approximately $2,930,000 for distribution to the facility. The Supply Warehouse also issued 41,262 bags of animal bedding valued at $309,390 and 7,808 bags of animal feed valued at $80,734. During this same period, 19,960 boxes of dry ice were issued, valued at $120,191, along with 111,300 gallons of liquid nitrogen valued at $40,068.

The annual physical inventory for the Central Supply Warehouse was conducted this year and involved the inventory of over 298 product valued in excess of $715,000, with a net adjustment realized of only -$356.

During this same period, the Maintenance Supply Warehouse received 5,788 requisitions containing 12,010 lines for materials and supplies valued at approximately $554,069 for issuance to FME craftsmen for the facility.

The annual physical inventory for the Maintenance Supply Warehouse was conducted this year, and involved the inventory of over 3,603 products valued in excess of $654,000, with a net adjustment realized of only -$103.03.

FME management transferred the shop stock items that were located in the various shops to the Maintenance Supply Warehouse along with new items being added on a regular basis. To date, over 550 items valued at just over $90,600 have been added.

Receiving/Delivery Section: The C&A Logistics Support Department (LSD) is responsible for maintaining a central receiving and delivery operation for all materials, supplies, and equipment ordered and delivered to NCI at Frederick. It is through this system that accountable government property is identified and tagged before it leaves Building 1050.

During this period (March 26, 2016 to July 12, 2016), the Receiving Section recorded 53,067 parcels entering the receiving area. Also, the warehouse at ATRF recorded 8,014 parcels entering the receiving area, and 2,063 parcels were distributed from the supply warehouse to various sites at ATRF.

The Delivery Section recorded 14,374 perishable deliveries and 27,587 non-perishable deliveries during the same period. Perishable items include chemical and biological materials typically required to be maintained in a frozen state and transported in dry ice containers. Non-perishable items include all dry materials, supplies, and equipment.

The Delivery Section has also recorded 608 LN2 cylinder deliveries, 300 specialty gases deliveries, 12,100 bags of animal bedding, and 2,397 bags of animal feed deliveries during the same period. The delivery metrics represent both the immediate turnaround of items recorded through receiving as well as draws from the Central Supply Warehouse.

Transportation Section: The Transportation Section is responsible for packaging and provisioning shipments that are external to NCI at Frederick via commercial carriers. During this period, the following metrics were recorded: 267 domestic hazardous and 52 international hazardous shipments (includes chemicals/biologicals and/or blood-borne pathogens that require special handling and packaging), 2,173 domestic non-hazardous and 344 international non-hazardous shipments, 618 shipments using contracted couriers, and over

7,902 hand-carry packages. In addition, the department applied postage to over 15,306 pieces for issuance to the U.S. Postal Service for delivery.

MANAGEMENT SUPPORT DIRECTORATE

Contract Administration Support

The Contract Administration Support Office provided coordination and assistance on activities specific to Leidos Biomed directorate/program needs and also responed to requirements from NCI programs. Management and interfacing on NCI at Frederick policies and procedures, standard processes, and standard operating policies were continued in support of NCI and contractor programs. Data call requests and management of responses were coordinated within required timelines. Operational questions from NCI and Leidos Biomed programs were managed to ensure responses, or, if required, to ensure that changes in practices were implemented in a timely and efficient manner. Monthly meeting with the NCI Office of Scientific Operations (OSO) focused on specific questions and activities in support of NCI at Frederick. Examples include user committees, conference facility support, conference room audio visual technical upgrades, and coordination of support and service level agreements for various support activities. Technical project management was provided in the transition of the Computer and Statistical Services Prime Contract with NCI to a subcontract relationship with Leidos Biomed. Representation on various committees such as the Information Technology Oversight Committee (ITOC), Accessioning System, Laboratory Animal Sciences Program (LASP) facility, Maximo, ATRF Operations Team, and Emergency Preparedness Committee are examples of the cross-functional support provided to both Leidos Biomed and NCI. Management guidance and oversight for the departments and functions within the Management Support Directorate (MSD) were provided to ensure individual and joint MSD efforts were coordinated and managed in an effective manner in support of the NCI at Frederick mission.

Project Management Office

The Project Management Office (PMO) continued to support and promote the "community" of project managers across the Leidos Biomed directorates to enhance communication and coordination between directorates, as well as between members of the senior management team and directorates, and to foster the application of sound project management principles. The PMO provides leadership for the implementation, improvement, and expansion of project management processes across Leidos Biomed by providing resources, tools, and templates to facilitate these processes. The PMO maintains and distributes recommended reading materials, and provides facility-wide training opportunities in areas of interest, including risk management and best practices for managing projects.

The MSD PMO continued to work closely with the Project Management Operations Office (PMOO) to support the implementation of Leidos Biomed's project management policy. Support included working with the Leidos Biomed programs to identify "projects" within their groups to which the policy would apply, participation in the in-progress-reviews (IPRs), reviewing and providing input to documentation and systems supporting the policy, providing training on the policy, and promoting the policy across the organization. Additionally, the MSD PMO worked with the PMOO to identify training topics in support of the policy.

Project Management Resources to Support the Needs of Programs

The PMO provided project and program management support for the:

***Physical Access Control System (PACS) Project*:** The PMO is leading the integrated project team responding to the Division of Personnel Security and Access Control's (DPSAC) request that the PACS at the NCI at Frederick be integrated with NIH's Quantum Secure to communicate with the NIH Enterprise Directory (NED).

Accessioning System Consolidation Project: The program manager is responsible for coordination of the overall planning and management within the context of the Accessioning System Consolidation Project requirements associated with the Operations and Technical Support (OTS) contract. The program manager interfaces directly with the project sponsors and Steering Committee members to ensure the Accessioning System Consolidation Project objectives and requirements are being executed.

FNLCR Business Systems Working Groups: Working groups were established to provide users of the various systems a mechanism to meet with back office and system support staff that support the business systems at FNLCR and address any user issues, customer issues, and receive system update information. Working groups provide users of the Enterprise Resource Program (ERP) systems a mechanism to meet with the reporting team to address any user issues, customer issues, and address additional project controls and report requirements. The PMO provides leadership for these meetings and manages action items generated from the groups. The groups meet on a quarterly basis.

Project Management Tools, Training, and Information

Regarding project management tools, training, and information, the PMO accomplished the following:

- Continued to facilitate Project Management Training by providing seven full-day seminars on project management:
  - Not-So-Secret Tools for Efficient Productive Project Management
  - Building Critical PM Skills: How to Make a Difference
  - Project Management Measurement Training
  - Request for Proposal (RFP)/Statement of Work (SOW) Development Training
  - Work Breakdown Structure (WBS) Milestone Management Training
  - Understanding and Capitalizing on Benefits of an Agile Approach
  - Project Management Essentials (two days)
- Conducting a monthly "Lunchtime Seminars" series focusing of project management techniques.
- Submitted Insite articles on project management to heighten awareness of the importance of sound project management.

NCI at Frederick Project Management Website Continued to maintain the NCI at Frederick Project Management website with the most current information regarding project management training (onsite and online), tools, templates, and project management library resources.

Quality Management Office

The Quality Management Office (QMO) integrates quality assurance activities with business process documentation and internal business and operational communications to ensure that documented procedures are current and that information included in these documents is communicated to the appropriate audience in a timely fashion.

Quality Assurance: QMO continued to work with NCI at Frederick OSO to coordinate the review and approval of operational goals and objectives for each six-month performance period of the contract year. Following the established process and associated timeline, SMART goals were approved before the start of each performance period. QMO prepares monthly status reports so that Leidos Biomed senior leadership is apprised of the status of all goals throughout each performance period. The QMO prepares a report on the final status of each goal that is provided to the NCI as an attachment to the semi-annual Contract Performance Status Report (CPSR).

QMO continued to manage a Performance Monitoring and Remediation Program to track the resolution of performance issues identified by both Leidos Biomed staff and their customers. In addition to monitoring how specific performance concerns are being addressed, outcomes of this effort include: lessons learned that can be shared across the organization, continuous improvement of operating procedures, and the development of metrics to evaluate performance.

Business Processes and Documentation: QMO continues to manage the review and revision of NCI at Frederick policies and procedures (P&Ps) and FNLCR standard processes (SPs). This effort includes ensuring that new and revised P&Ps and SPs are regularly reviewed, communicating changes to Leidos Biomed managers, and making current versions available to all Leidos Biomed staff. The QMO initiated a restructuring of the NCI at Frederick P&P manual to convert P&Ps into high-level policies by moving process-related information to supporting SPs or standard operating procedures (SOPs). This approach creates a policy manual with fewer entries that applies to both government- and contractor-operated components of NCI at Frederick. The process of converting Leidos Biomed and NCI at Frederick forms to the new standard format continued during the year. Revised forms are being provided in an electronically fillable format, with detailed instructions, and made available in a central location accessible through the NCI at Frederick website.

The Laserfiche Enterprise Content Management platform continued to be used to manage the creation, retrieval, and storage of records within the Operations and Financial Groups. QMO and Information Systems Program (ISP) groups provided technical and administrative support for the Laserfiche platform, including the administration of over 200 user accounts.

QMO continued to coordinate efforts between the vendor, NCI, and Leidos Biomed program areas to facilitate the transfer of records between NCI at Frederick and off-site storage facilities as well as the destruction of records that no longer need to be retained. In addition to hard-copy records, QMO is also coordinating the off-site storage of data stored in electronic format.

Business and Operational Communications: Regular communications to Leidos Biomed employees continued to be managed by QMO. These communications are for the purpose of notifying all managers and supervisors of changes to NCI at Frederick P&Ps and FNLCR SPs and forms, and explaining the operational impact of these changes.

The QMO continues to manage the preparation of the Leidos Biomed Annual Report on operations and accomplishments at the FNLCR. The QMO also participates and provides oversight for the preparation of semi-annual Contract Performance Status Reports in support of the OTS contract Performance-Based Award Fee Plan.

Microsoft (MS) SharePoint tools continued to be deployed to provide NCI and Leidos Biomed directorates with a common platform to collaborate on major projects and initiatives. MS SharePoint supports team collaboration on projects and features a secure central document repository with document version control. MS SharePoint sites and subsites are established and maintained by QMO and Business Information Systems Group (BISG), which now maintain approximately 400 individual sites.

Public Affairs Office

The Public Affairs Office (PAO) supports the mission of the FNLCR through a broad spectrum of outreach activities to engage constituents at the local, state, and national levels. The office informs key audiences about the national lab's scientific accomplishments, national importance, and local community impact.

Significant Achievements

The office increased public understanding and appreciation of the national laboratory's scientific accomplishments through interactions with the news media; by direct reporting on Poster, Insite, and frederick.cancer.gov; through social media; and by working with research partners, collaborators, subcontractors, and professional organizations on the public release of information.

A special effort has been made to increase the quantity and quality of coverage from local news organizations, particularly the *Frederick News-Post*. This effort has included periodic meetings with the editor of the paper and regular contact with the news staff. These activities have begun to pay off in a significant increase in the number and quality of articles, giving both the NCI at Frederick and the FNLCR greater visibility in the local community and beyond.

Among major topics covered by the local media: the AIDS and Cancer Virus Program's work in uncovering hidden pockets of human immuno-deficiency virus (HIV) that, once revealed, can be targeted for potential eradication; the Biophysics Section of the Center for Cancer Research and facilities in Frederick that are advancing progress in imaging biological structures using cryo-electron microscopy; the collaboration of NCI and the Department of Energy on exascale computing and applications of the proposed capability in biomedical research, with the RAS Initiative as one of the pilot projects; the appointment of a new chief operating officer for the FNLCR; and a collaboration between the Nanotechnology Characterization Laboratory (NCL) and eight European partners to launch an NCL-like operation in the European Union.

The PAO has also worked with other collaborating institutions and subcontractors in delivering important institutional messages to the public via news releases, blog posts, website pages, and through broadcast and social media. A noteworthy example is the recent news release and announcement by Vice President Joe Biden calling attention to the NCI's Genomic Data Commons being managed at the University of Chicago under a research subcontract from Leidos Biomed. Other examples: subcontractor Aphios' news release on developing a nanoformulation of its anticancer drug; news releases by 11 of the 22 institutions chosen to participate in the expanded Chemical Biology Consortium, the discovery engine of NCI Experimental Therapeutics Program (NExT); a statement for release by the White House Office of Science and Technology Policy about the value of citizen science programs; and Discovery Channel's extended coverage of a clinical trial supported in part by Leidos Biomed staff stationed at the NIH Clinical Center.

The PAO has contributed to an increase in science coverage by the Leidos Biomed employee news site, Insite, and via the NCI at Frederick news site, the Poster, and redistributed those articles via Facebook, Twitter, email, and at community (i.e., Frederick County Chamber of Commerce Business Exposition) and national networking events (i.e., BIO International Conference). Selected topics: targeting hidden sanctuaries of the AIDS virus, combatting human papillomavirus (HPV)-related oral cancers, targeted gene therapy, collaboration with big pharma to get nanotechnologies into early-stage drug development, new treatment for children with neuroblastoma, RAS investigator retreat, science education and student mentoring, advances against Middle East Respiratory Syndrome (MERS), genetically modified soy beans that produce an anti-AIDS ingredient, and a natural product demonstrating effectiveness against colon cancer.

The office has provided guidance for staff on branding issues to ensure consistency in presentation, both inside and outside of the organization, of the new, integrated NIH logo and NCI branding guidelines, as well as differentiating the FNLCR as government-owned, contractor-operated.

The PAO has played a major part in the ongoing effort to update and revise frederick.cancer.gov so we have an effective public platform for demonstrating excellence in science, offering business and collaborative research opportunities to outside organizations, supporting the recruitment and retention of a highly skilled and productive contractor workforce and the general sharing of knowledge and resources consistent with the role of a national lab.

The PAO continued its contributions to the Poster and its guidance of Insite, with coverage of the organization's scientific accomplishments, employee achievements, institutional departments, and volunteer outreach activities. Related social media platforms further disseminated information on scientific accomplishments and employee achievements. This effort also gave additional visibility for job opportunities, active procurement and subcontracting solicitations, and community engagement.

Events, presentations, and on-site outreach activities for the year included: Frederick Day in Annapolis, the Frederick County Chamber of Commerce Business Expo, annual meeting of the American Association for the Advancement of Science, BIO International Convention, In the Street festival, Spring Research Festival, Frederick County Annual Business Reception, NCI Principal Investigator retreat, Frederick County Community Cancer Coalition, the Fort Detrick Public Affairs Roundtable, and Fort Detrick Alliance meetings and events. The office directly sponsored (via corporate funds) and collaborated with other organizations in community engagement and fundraising events such as those involving the Mission of Mercy, Habitat for Humanity, American Cancer Society, Frederick Memorial Hospital and its new cancer center, Mid-Atlantic Hispanic Chamber of Commerce, Frederick Community College, CREST, Literacy Council of Frederick County, Blessings in a Backpack, Community Foundation of Frederick County, United Way of Frederick County, Hospice of Frederick County, Alzheimer's Association, and the Children's Inn at NIH.

The public affairs director continued to represent the interests of the national lab as a board member of the Fort Detrick Alliance. The director served on the review committee for the Leidos Biomed–sponsored Michelle Shearer STEM Scholarship Fund, administered by the Community Foundation of Frederick County, and led the annual contractor employee giving campaign, which contributes to community nonprofits supporting education, workforce development, health care, and charity.

Scientific Publications, Graphics & Media

Scientific Publications, Graphics & Media (SPGM) continues to meet the needs of the research community through a wide array of editorial, creative, and production services.

The demand for editing scientific manuscripts continued to rise. From July 2015 through July 2016, the SPGM Editorial Office edited 47 manuscripts, compared with 45 in the previous period. SPGM editors and illustrators provided support for the Gene Regulation and Chromosome Biology Laboratory (GRCBL) site visit, while the editors continued to write articles for numerous major scientific journals and the two online newsletters (Insite and Poster).

SPGM saw significant changes in the editorial office over the last year. The editorial supervisor position changed hands twice during this period and the staff is now comprised of all new hires. The third open position in editing was filled July 2016 with a start date in August.

SPGM publishes two online newsletters, the Leidos Biomed newsletter Insite and the NCI at Frederick Poster. Both newsletters continue to show increases in readership and garner positive reactions from subscribers. The newsletters' main focus is on the research within the NCI at Frederick community.

Demand for scientific illustration has continued to increase, as well. Notably, SPGM illustrators' work appeared on journal covers including the ACS Chemical Biology Journal for April 2016 (#4). SPGM illustrators created materials for the NCI RAS Initiative Symposium, including name badges, table tents, parking signs, and map graphics. SPGM produced and printed the scientific poster for the event and provided photography services during the event. SPGM continued to support the Ebola PREVAIL flip books. SPGM has updated and modified publications to meet the needs of the National Eye Institute and National Institute of Neurological Disorders and Stroke. SPGM illustration contributed support to the 2015 Frontiers in Basic Immunology Meeting, sponsored by the Center of Excellence at the Center for Cancer Research (CCR), NCI. SPGM produced flyers, web banners, name badges, postcards, programs, abstract books, and onscreen show screen warmer slides and templates. SPGM illustrators continue to produce the CCR *Connections* newsletter.

The SPGM photographer was busy with an increase in the number of photo assignments from in-house publications and from groups and individuals seeking group and portrait photos.

Poster production has leveled off and is consistent with last year's production levels, while video production significantly increased, with several important meetings and numerous interview videos for the Neuro-Oncology Branch (seven), the Division of Cancer Epidemiology and Genetics (three), and the Genomic Data Commons (three), including the creation of motion graphics by SPGM illustrators.

SPGM has expanded its email listserv notices and will continue to demonstrate its capabilities to the research community through periodic email distributions.

SPGM is beginning work with CCR on its Research Highlights booklets and setting up for the second Contractor Performance Status Report of the year, followed by the Leidos Biomed Annual Report in the fall.

Operational Support

Travel

The purpose of the Leidos Biomed Travel Department is to coordinate travel arrangements for business travel required by scientific and administrative employees. Travel is requested by program areas to attend trainings, participate in scientific collaborations, assist with clinical studies, or attend scientific meetings and seminars. The Travel Department obtains the appropriate approvals and coordinates arrangements for event registrations, flights, hotel, and ground transportation for employee travelers.

The workload within the Travel Department continues to grow, particularly with the increase in overseas clinical trial support within the Clinical Monitoring Research Program (CMRP). Between September 26, 2015 and July 21, 2016, the Travel Department coordinated travel arrangements for 716 trips. The Travel Department is currently staffed with 1.5 full-time equivalent (FTE) employees and continues to establish business processes for coordinating travel more efficiently.

Conference and Event Planning and Coordination

The Conference and Event Planning Department plans and coordinates U.S. Department of Health and Human Services (HHS) conferences, symposiums, workshops, and other administrative meetings for programs affiliated with the FNLCR.

The department manages a wide range of responsibilities related to the logistics of an event including finding/obtaining appropriate meeting space, contract negotiations, database management to include establishment of websites for registration, lodging and travel arrangements for participants, and supervising selected meeting functions at the event location. The coordination and management of the travel arrangements for sponsored attendees and speakers is one of the key functions of the Conference and Event Planning Department.

The department managed 67 conferences, symposiums, and events during contract year 2016. In total, these meetings were attended by over 8,600 registered participants, which included more than 1,000 sponsored attendees/speakers whose travel arrangements were supported through the department. In addition, the department was responsible for overseeing over $1.1 million worth of budgets for the various conferences and events.

Conference Centers

The NCI at Frederick Conference Center staff is responsible for the planning, testing, setup, and execution of conferences and meetings at both the ATRF and NCI at Frederick campus.

The Conference Center staff maintains audio-visual (A/V) equipment (such as video teleconference [VTC] units, projectors, laptop computers, flat screen TVs, microphones, and speaker systems) in 63 conference rooms at the ATRF and the NCI at Frederick. The Conference Center staff also manages major A/V technical refreshes of all conference rooms and maintains an A/V and VTC strategic plan that lays out the timeframe and plan for recurring upgrade efforts.

For contract year 2016, over 2,300 meetings/events were held at the Building 549 Conference Center, and over 1,650 meetings/events were held at the ATRF Conference Center. For those meetings, the Conference Center staff established over 370 VTC connections at the Building 549 Conference Center, over 80 VTC connections at the ATRF Conference Center, and over 90 meetings using the WebEx application. Meetings can range from recurring one-hour basic meetings to multiday events. Events such as symposiums, workshops, town hall meetings, retreats, and other committee-style meetings may require project planning, intensive technology testing, and other resource challenges.

HUMAN RESOURCES

The Human Resources Directorate (HR) works in partnership with managers and their teams to support the mission of Leidos Biomed staff as they work on behalf of the FNLCR.

Human Resources provides leadership and guidance in the development, implementation, and equitable administration of policies and procedures, thus fostering a positive work environment. The core services and competencies include recruitment and staffing, employee relations and counseling, organizational and employee development, compensation and benefits, HR information management, and regulatory compliance.

Workforce Demographics

A review of Leidos Biomed demographics shows that the contract year-end employee population was 1,972. Within this total population, 54.46 percent are female and 31.54 percent are minority. Annualized voluntary turnover was 8.7 percent for this period. This turnover level continues to compare favorably with the marketplace. Annualized involuntary turnover related to performance was 1.8 percent for this period.

Recruitment

HR continues to provide specialized recruitment strategies in response to the unique hiring needs of each program. During the past year, 544 positions have been filled; of these, 66 percent were exempt positions and 34 percent were nonexempt positions. Average recruitment time (between the date of the job posting and the accepted offer) was 52 calendar days, and the average time for a candidate to start work from date of acceptance was 30 calendar days.

The Talent Acquisition (TA) staff continues to utilize the current resources to network and source highly qualified candidates. We met with the representatives from niche websites and mainstream job boards to analyze the current targeted advertising strategy, review return on investment, and develop a plan for a 2017 social media and branding/rebranding of FNLCR/Leidos Biomed. As a result, we can now monitor and ensure consistent usage from recruiters in our resume databases and begin using analytic tools like CareerBuilder's Recruitment Edge. We initiated a strategy to reinvest in a niche site (Association of Clinical Research Professionals [ACRP]) to post critical skill needs and, in parallel, ran a trial period using their database to source members. A TA team member represented Leidos Biomed at the Oncology Nursing Society Conference to brand, network, and source a host of passive job seekers. TA staff members participated in career events, including those specifically targeting military, diversity, and college, for specialty positions in clinical and information technology fields. The new TA manager worked to establish a relationship with the Frederick County Workforce Services, Dept. of Veterans, Division of Rehabilitation Services, and Frederick Community College to partner on various outreach initiatives in the community and network with future job seekers. During this period, we were also able to fill the vacant recruiter roles and have begun to distribute and optimize the requisition load of each recruiter averaging 20 open positions.

Training

Human Resources conducted employee courses on Delivering Effective Presentations, Managing Up–How to be Effective with Your Boss, Effective Business Writing, and Influencing Without Authority. A webinar was conducted for New Manager Performance Review Training. In April 2016, we began the ManageWell Program, an internal leadership development certificate program provided for newly hired or promoted managers that offered essential relevant topics needed to successfully manage employees and teams. The program consisted of eight interactive course modules that must be completed to received completion certificates.

Compensation and Benefits

Completion of the 2017 health insurance renewal process resulted in an overall premium increase of less than 3 percent. A new flexible spending vendor was selected, resulting in lower monthly administrative maintenance fees.

Employee Relations and Retention

Employee relations staff supported the reduction in force and provided outplacement material to the 25 employees who received notification. Employees were provided one-on-one job search outplacement support.

Personnel

The Operations Group consists of 349 employees in professional, administrative, clerical, project management, customer services, and operations positions.

Operations Group	# of Employees
Contracts and Acquisitions	105
Financial Operations	40
Human Resources	19
Management Support	20
Operations	6
Facilities Maintenance & Engineering	159
Total	**349**

FINANCIAL OPERATIONS DIRECTORATE

The Financial Operations Directorate (FOD) oversees all finance-related activities for the Frederick National Laboratory for Cancer Research (FNLCR), including the following functions: general accounting, payroll, accounts payable, billing, accounts receivable, financial planning and analysis, core business information systems, and audit and compliance. The directorate's mission is to administer an enterprise-level, integrated financial management program to support the full mission of the FNLCR; establish fiscal policies that ensure accountability for, and control of, government funds; provide timely, relevant, and clear financial analysis and reporting to assist in managing fiscal resources; and deliver accurate, timely, and complete financial information to FNLCR stakeholders.

During the past contract year, the FOD team implemented a significant restructuring of its cost accounting practices to support the new multi-contract environment of the FNLCR. To maintain compliance with applicable federal regulations and standards, FOD adopted an indirect rate structure to enable the allocation of indirect cost across multiple contracts.

FOD reinitiated the Finance Working Group forum between Leidos Biomed and key government stakeholders (Management Operations Support Branch [MOSB] and Office of Scientific Operations [OSO]). These meetings were held on a biweekly basis and provided an effective forum to facilitate communication and interaction on FOD-related priorities and initiatives to align both legacy and emerging support requirements to FNLCR stakeholders.

Controller's Office

The **General Accounting Department** performed a broad range of activities under each of the following categories: provided corporate interface for financial reporting and policy compliance issues; maintained daily activity of the Special Bank Account (SBA); processed accounts payable; processed billings and receivables, including preparing contract compliant cost-incurred invoices on a biweekly basis; ensured compliance with all statutory and regulatory legal requirements related to accounts payable and payroll (including monitoring Service Contract Act (SCA) average benefit rate compliance); and forecasted and monitored fringe, general, and administrative indirect costs and rates.

The **Accounts Payable Department** performed invoice processing for vendor and subcontractors and addressed vendor inquiries related to payment. Interaction with purchasing, receiving, contracts, and program organizations are performed proactively, on a daily basis, to ensure operational effectiveness and process efficiency. All vendor invoices are received at a processing center and transmitted and approved electronically, thus eliminating the need for manual entry and filing.

Significant Achievements

The Controller's Office certified Sarbanes/Oxley Act of 2002 (SOX) cash controls each quarter, effectively administered the SBA and closely monitored funding requirements, finalized the CY2015 fringe rate, and established a new provisional fringe rate for CY2016. The accounting department was an integral partner in the development and implementation of cost accounting changes and the adoption of the indirect cost pools and related indirect rates. Changes to all impacted enterprise resource program (ERP) systems were tested and implemented in a very compressed timeframe to maintain operational performance and readiness of the financial systems.

In addition, accounting has responded to numerous data request and inquiries from NCI with regard to invoiced costs on both the Operations and Technical Support (OTS) and indefinite delivery/ indefinite quantity (IDIQ) invoices. Additional supplemental reports were developed for submission to NCI as invoicing backup, and multiple meetings were held with NCI to clarify how the indirect rate pools are applied and billed. Accounting provided invoice copies and travel detail in response to audit inquiries from NCI.

In support of the weekly Finance Working Group meetings, the accounting department prepares and submits SBA cash forecast analysis and an invoice aging report and payment metrics for discussion.

Financial Planning and Analysis

The Financial Planning and Analysis (FP&A) group provides support for cost management, budgetary development, and coordination of fiscal processes to the NCI at Frederick community. For the current reporting period, we have monitored about 14,400 individual projects including annual tracking operating costs and associated funding levels for the OTS contract of approximately $687 million. In addition, FP&A directly supported the proposal pricing, award, and implementation of 18 new task orders (TOs) awarded under the IDIQ contract; these FY2015 TOs had an awarded value of approximately $200 million. FP&A is now actively supporting the tracking of costs, ceilings, and associated program funding for all awarded task orders.

Significant Achievements

During FY2016, the FP&A office has been involved in the following major activities:

- Successfully developed and implemented an indirect rate structure for OTS and IDIQ contracts, including forward pricing rate submission, and updated the contractor's Cost Accounting Standards Board (CASB) Disclosure Statement.
- Provided multiple analyses for OSO on implementation and interpretation of indirect rates and their impact on NCI budget and funding.
- Actively coordinated the redesign of standard financial reports and budgets to accurately and transparently incorporate the financial impacts of the adopted cost accounting changes and the addition of the new indirect cost burdens.
- FP&A was part of the Leidos Biomed team responsible for pricing and award of 18 TOs under the new IDIQ contract. This involved developing a pricing template, working with program areas on the labor portion of TOs, application of indirect rates on TOs, and finalizing the total pricing file for inclusion in TO awards. This was completed under an extremely tight timeline in order to meet NCI year-end funding schedules. To assist in monitoring/projecting cost for the TOs, FP&A has developed a TO quarterly reporting analysis that takes into consideration life cycle, including cost-ceiling management and Limitation of Cost requirements.

- Collaboratively participated on an internal team that developed new Basis of Estimate and pricing templates to support the FY2016 Task Order Request for Proposal response process. In addition, FP&A worked with the Project Management Office (PMO) to accommodate the addition of cost/schedule/performance requirements through milestone identification and pricing in response to additional contractual requirements identified by the government.
- Provided costs-to-go analysis on a monthly basis for close-out and deobligation of unused FY2011 funds. Worked on an integrated team of contractor and government stakeholders to align funds for approved efforts to ensure maximum utilization of remaining funding.
- Developed a new project close-out process that considers all aspects of systems that provide information to financial reports to ensure no additional cost will be assigned to project closing. Established a watch list so new attempts to use project ID will be flagged and notified that the project is in process to close.
- Developed a new monthly Center for Biomedical Informatics and Information Technology (CBIIT) subcontract review process to facilitate more precise research contract estimates-at-completion (EACs) to assist with overall division financial management and funding analysis.
- FP&A staff supported the Laboratory-Directed Exploratory Research (LDER) team to develop and implement the second year of this research program at FNLCR.
- Prepared Limitation of Funds analysis on total contract funding for submittal after each funding mod. With limited funding opportunities, it is imperative to understand how long the current funding by appropriation will last. Worked with OSO and MOSB to ensure adequate funding was added to the contract to continue to deliver service.
- FP&A has been more intimately involved on the funding side, with requirements to monitor to available funding levels and funding expirations as well as monitoring to annual budget. This includes approving research subcontracts funding and period of performance, advising on potential staffing actions, estimating funding requirement for specific contract funding periods, including funding on monthly reports, evaluating division use of funds prior to expiration of severable funding, etc.
- Provided extensive support to financial reviews, discussions, monitoring, reporting, and analysis of the repository subcontract to assist in financial oversight of the budget and cost management of this subcontract.
- Provided monthly/quarterly cost management/ status reports for various high-profile projects/ divisions (i.e., Vaccine Research Center, Biopharmaceutical Development Program; Office of Cancer Genomics; Division of Cancer Epidemiology and Genetics; agencies; NCI CBIIT; Office of the Director, Center for Strategic Scientific Initiatives; Office of the Director, Immediate Office of the Director; and NCI Experimental Therapeutics [NExT]), which are used by the customer to make financial decisions on the future operations of each of these programs.
- **Contract data requirement lists (CDRLs) prepared and delivered:**
  - Will submit comprehensive FY2017 proposed budgets for all divisions and programs, including new life cycle budgets for non-severable and TO projects, by contract deadlines – September 1
  - Provided Annual EAC
  - Provided quarterly funding/cost updates to NCI to reflect severable/non-severable tracking requirement
  - Provided quarterly budget revision report
  - Provided quarterly AIDS cost and budget report
  - Provided quarterly Division of Cancer Epidemiology and Genetics (DCEG) Progress report
  - Provided annual Core Service Pricing

Internal Audit

The Internal Audit group accomplished the following during the reporting period:

- Identified $1,313,541.31 in cost disallowances for the period.
- Audited cost-reimbursable subcontracts to verify compliance with Federal Acquisition Regulation (FAR) Parts 31.201, 31.201-3, and 31.201-4.
- Audited time-and-materials subcontracts to verify compliance with FAR Part 16.601.
- Performed desk audits of 14 research subcontracts in preparation for contract close-out (including final American Recovery and Reinvestment Act [ARRA]-related subcontracts).
- Initiated a timesheet audit process that includes labor interviews to verify compliance with time-charging policies.
- Audited transactions related to 401(K) and defined benefit plans.
- Audited 2,200 expense reports for compliance with Federal Travel Regulations (FTR).

Business Information Systems

The **Business Information Systems Group (BISG)** provides management reports for the directorates and government programs to assist with cost management; application support, user setup, and security for the ERP financial system applications;

application administration and helpdesk support to the Time and Attendance system for Leidos Biomed personnel; application support to SharePoint and to Facilities Maintenance and Engineering's (FME) computerized maintenance system; and performs business process analysis in order to design and develop workflows that will create efficiencies across FNLCR.

Core Business Systems Supported by BISG

- Cognos Reporting
- Cognos TM1
- CostPoint
- Travel and Expense
- COR360
- Hyland OnBase (Docs for Deltek)
- SharePoint
- Maximo
- Project Setup
- Unanet
- Laserfiche

SIGNIFICANT ACHIEVEMENTS

- Modification to existing suite of standard financial reports to reflect the new cost structure and incorporate the new indirect rate burdens on projects and programs.
- Assisted FP&A with the development of new budget templates to support budgeting of severable and indirect budgets using the TM1 platform, including the development of new budget reports using Cognos. This initiative also yielded a new budget tool that enables full life-cycle (multi-year) budgeting and forecasting on all active and ongoing Non-Severable programs.
- Restructured current licensing of Cognos to facilitate a growing user base while capturing net cost savings on future service maintenance agreements (estimated cost savings of approximately $199,000 anticipated between 2016 and 2020).
- Worked with FP&A to design, develop, and deploy a database to support pricing of new Task Order Request for Proposal (RFP) responses under the IDIQ contracts.
- Worked with Chief Operating Officer to procure Jive software to launch a collaboration platform for Leidos employees to enhance communication and engagement yielding propagation of information sharing and best practices.
- In coordination with the Controller's office, BISG developed additional supplemental billing reports to support additional invoicing requirements requested by the government.
- Developed a project plan for the testing of the CostPoint 7.1.1 upgrade, including development of a comprehensive set of test scripts for each back-office group and development of a master project schedule.
- Worked with Centeva (contractor to NCI Office of Acquisitions) to facilitate a holistic reconciliation of NIH business systems (government) to Leidos (contractor) funding and cost data for the full life cycle of the current contract.

CENTRAL GLASSWARE SERVICES

Central Glassware Services consists of five processing kitchens and a daily van run that provides satellite services to 18 buildings.

During FY2016, Central Glassware provided glassware processing services to 241 laboratories at NCI at Frederick. Services include the daily pickup and processing of soiled glassware and the restocking of sterilized glassware. Central Glassware also provides special services on request, including washing velvets, preparing bell units, preparing pipettes, processing specialized glassware, autoclaving liquids, processing laboratory spatulas and stir bars, and transporting and delivering media. All used media bottles and caps are recycled.

SIGNIFICANT ACHIEVEMENTS

During this reporting period, Central Glassware added support to 14 new laboratories in the Advanced Technology Research Facility (ATRF) building, three new laboratories in the 539 building and three new laboratories in the 560 building. Central Glassware provided services (via the van run) to Buildings 321, 376, 426 OHS, 431, 433, 469, 535, 538, 539, 550, 560, 562, 567, 1036, 1047, 1066, 1071, and the Advanced Technology Research Facility (ATRF). Centralized ordering and delivery are accomplished through the van run.

Central Glassware continues to provide media transport services to and from Building 539 (via van pickups) to 24 laboratories in Buildings 469, 535, 538, 539, 567, and 560.

Material Processed Annually

Building	Material Processed
ATRF	120,900
535	165,000
538	78,780
539	206,580
560	223,000
Van Run Pickups	
321 (from 560)	3,290
376 (from 560)	60,420
426 OHS (from 560)	70
431 (from 538)	1,500
433 (from 538)	3,000
469 (from 538)	15,600
550 (from 560)	1,200
562 (from 560)	1,950
567 (from 560)	28,000
1036 (from 560)	3,900
1047 (from 560)	3,120
1066 (from 560)	6,750
1071 (from 560)	1,950
Total, material processed	**925,010**

Appendix A:
Company Overview

leidos

Leidos Biomedical Research, Inc.

Leidos Biomedical Research, Inc.

2015-2016 Annual Report

Appendix A

Company Overview

OFFICE OF THE PRESIDENT

David Heimbrook, Ph.D., President

Cancer Genomics Research Laboratory

Meredith Yeager, Ph.D., Senior Principal Scientist/Scientific Director

With remarkable advances in genomic technologies, the Cancer Genomics Research Laboratory (CGR) was established by the National Cancer Institute (NCI) to investigate the contribution of germline genetic variation to cancer susceptibility and outcomes in support of the Division of Cancer Epidemiology and Genetics (DCEG). Working in concert with epidemiologists, biostatisticians, and basic research scientists in DCEG's intramural research program, the CGR provides the capacity to conduct genome-wide discovery studies and targeted regional approaches to identify the heritable determinants of various forms of cancer. CGR supports DCEG in all stages of cancer research from planning to publishing, including experimental design and project management, sample handling, genotyping and sequencing assay design and execution, development and implementation of bioinformatic pipelines, and downstream research and analytical support.

Currently, the CGR utilizes a variety of technology platforms to assess human genetic variation. Platforms and technologies include: (1) single-nucleotide polymorphism (SNP) detection via TaqMan™ fluorescent 5' nuclease cleavage with detection on the Applied Biosystems 7900; (2) Illumina bead-based SNP array multiplexing technologies, both for genome-wide discovery and targeted custom analysis; (3) Illumina genome-wide methylation arrays; (4) relative telomere length analysis; (5) sequencing of large genomic regions, including whole exomes, as well as targeted sequencing and analysis using five platforms (Illumina HiSeq2500/4000, Ion Torrent Personal Genome Machine (PGM), Ion Torrent Proton, Illumina MiSeq, and Illumina NextSeq 500) in conjunction with a wide range of sequence capture and targeting methods and instrumentation.

CGR also includes the DNA Extraction and Staging Laboratory (DESL). This highly automated laboratory is responsible for all DCEG specimen preparations, including nucleic acid extractions, sample staging, generation of run-ready plates for genotyping and sequencing applications within CGR, and specimen aliquoting for use by collaborators around the world.

It would not have been possible to manage the laboratory work, project planning, quality control, assay validation, sample handling, and data analysis without the support of the staff members organized across CGR's eleven functional groups: DESL, the Production Laboratory, the Scientific Research Group, the Functional Research Group, Technology Implementation, Quality Assurance/Quality Control (QA/QC), LIMS Development, Bioinformatics, IT Core Services, Project Management, and Administration.

Project Management Operations Office

Kathy Terlesky, Ph.D., Director

The Project Management Operations Office (PMOO) entered its second year of operation rolling out the project management framework across the organization. Organizations performing project-based work were identified, and directorate heads and project managers were trained on the system. The system includes the In-Process Review SharePoint site for project execution oversight, and methodology and templates for project execution. The goal of the new project management policy is to create a project management culture within Leidos Biomed that is flexible but disciplined and is more closely aligned with industry best practices. Similar to the way that the Yellow Task tracking system highlights issues with respect to project initiation, we expect improved project management systems to highlight issues with project execution and facilitate rapid resolution of those issues. In the current reporting period, there were 49 in-process reviews held with executive leadership and 4 training sessions serving over 70 participants. The reviews give executive leadership visibility into projects across the diverse customer set and the venue to provide targeted solutions to issues or risks the project managers were facing. The project management framework was first applied to all FY2015 non-severable task orders followed by all non-severable projects from prior years.

The PMOO also manages the Yellow Task System. In the current reporting period, there were 67 severable Yellow Task Requests of over $56 million in value received from 17 different divisions, offices, and centers. The requests were roughly split between customers from the intramural community and the extramural community. The requests were for a variety of needs including programmatic support, position support, reduction request, repository request, and laboratory support requests.

The staff of the PMOO supports surge data and programmatic requests as required. In the current reporting period, this has included the Cost-to-Go effort to proactively find ways to complete scope on closing FY2011 non-severable Yellow Tasks. The PMOO-led team was able to decrease the amount of money projected to be returned to the treasury from over $11 million to approximately $0.6 million. This was accomplished by working across the Leidos Biomed and government customer scientific community to identify current research needs and match them to the defined FY2011 non-severable research needs and incomplete projects. The concerted effort brought together over 25 NCI investigators to support sequencing of cancer specimens to increase the body of knowledge behind cancer genomics.

SCIENCE AND TECHNOLOGY GROUP

David Heimbrook, Ph.D., Chief Science Officer, Interim

Cancer Research Technology Program Directorate

Dwight V. Nissley, Ph.D., Director

The Cancer Research Technology Program (CRTP) leads scientific initiatives and provides technical solutions to the National Cancer Institute (NCI) and National Institutes of Health (NIH) to meet the challenges of and carry out mission-driven biomedical research. In conjunction with the NCI Office of the Director and Dr. Frank McCormick, a consultant recognized as an expert in the RAS field, the CRTP and Leidos Biomed developed and implemented, and the CRTP launched, the NCI RAS Initiative, which is now discovering therapeutic leads towards the goal of finding inhibitors of oncogenic RAS in RAS-driven cancers. The RAS Program continues to evolve and has implemented the RAS Program Hub-and-Spoke Model to amplify hub efforts for the benefit of the extramural research community. These efforts include collaboration between the FNLCR Hub, extramural NCI-supported labs, pharma, and intramural labs. These collaborations were initiated via partnerships facilitated through NCI and contractor mechanisms, including Material Transfer Agreements, Technical Services Agreements and Contractor Collaboration Agreements, and Cooperative Research and Development Agreements.

In addition, the CRTP continues to lead and support NCI efforts to enable the extramural research community through the Antibody Characterization Laboratory (ACL) and Nanotechnology Characterization Laboratory. The CRTP has also established dedicated laboratories to support ongoing research efforts in collaboration with CCR and DCEG. Over the past year, CRTP collaborated with CCR to establish The Center for Molecular Microscopy (CMM) and is working with Dr. Sriram Subramaniam and the FNLAC to establish a National Cryo-EM facility.

CRTP is composed of the following:

Dedicated programs:

- Antibody Characterization Laboratory
- Nanotechnology Characterization Laboratory
- CCR Sequencing Facility
- Center for Cancer Research Dedicated Core Services
- Center for Molecular Microscopy

RAS research:

- Validation of KRAS as a target (Project Zero)
- Structural and biophysical characterization of KRAS (Project 1)
- Identify compounds that inhibit KRAS-driven tumors (Project 2)
- Characterize and disrupt KRAS complexes and probe the nature of KRAS dimerization (Project 3)
- Cell-surface mapping (Project 4)
- Synthetic lethal screens (Project 5)
- RAS reference reagents (Project 6)

Core support to RAS:

- Electron Microscopy Laboratory
- Protein Characterization Laboratory

- Protein Expression Laboratory
- Genomics Laboratory
- Center for Molecular Microscopy

AIDS and Cancer Virus Program Directorate

Jeffrey D. Lifson, M.D., Director

The AIDS and Cancer Virus Program (ACVP) consists of both investigator-initiated research sections and research support core laboratories. During the review period, the research portion of the ACVP comprised the laboratories of five principal investigator (PI)-headed research sections pursuing independent yet related multidisciplinary research programs in basic or applied molecular virology, viral immunology, retroviral pathogenesis, and viral oncology. The scientific staff of the ACVP encompasses expertise in a broad range of scientific disciplines, and there is a strong tradition of collaboration between the PIs. The studies pursued by the laboratories have, as a unifying feature, their direct or potential relevance to the overall goal of developing an effective vaccine or other approaches for the prevention or treatment of HIV infection and AIDS, as well as relevance to the study of viruses involved in cancer. The program helps fulfill the mission of NCI by contributing to the advancement of our understanding of HIV and AIDS, a major cause of morbidity and mortality in the United States and around the world, and a predisposing factor for AIDS-associated malignancies. Through research on vaccines and other approaches for the prevention and treatment of HIV infection and AIDS, the ACVP also seeks to have a practical impact on this global problem. Finally, the ACVP seeks to add to the legacy of important contributions to AIDS research and viral oncology made by NCI scientists. The ACVP has been very productive over the last year, contributing to 57 articles in peer-reviewed journals, including multiple high-impact publications.

Basic Science Program Directorate

Mary N. Carrington, Ph.D., Director

The Basic Science Program (BSP) consists of both investigator-initiated research laboratories and personnel who work in support of the National Cancer Institute (NCI) Center for Cancer Research (CCR) laboratories. The research component encompasses laboratory sections of seven principal investigators (PIs), each of whom pursues independent, multidisciplinary research. The PI laboratories include: the Human Leukocyte Antigens (HLA) Immunogenetics Section, headed by Dr. Mary Carrington; the Molecular Immunology Section, headed by Dr. Stephen Anderson; the Molecular Immunotherapy Section, headed by Dr. Thomas Sayers; the Hematopoiesis and Stem Cell Biology Section, headed by Dr. Jonathan Keller; the Molecular Genetic Epidemiology Section, headed by Dr. Cheryl Winkler; the Computational Structural Biology Section, headed by Dr. Ruth Nussinov; and the Epigenetics Section, headed by Dr. Katherine Muegge. Researchers who are embedded in CCR laboratories are organized into four scientific sections: the Chemistry and Nanotechnology Section, the Cancer and Immunology Section, the Basic Research Section, and the Genetics Section.

BSP also provides services and products to support CCR's research efforts. The Cancer and Inflammation Program (CIP) Genetics and Microbiome Core carries out statistical and bioinformatics analysis in support of CIP investigators; the Fluorescence-Activated Cell Sorting (FACS) Core carries out flow cytometry and analysis for CIP and CCR scientists; the Media Laboratory produces media and reagents for more than 45 CCR laboratories; Central Glassware Services provides glassware processing to the laboratories; and the BSP program office provides logistical and administrative support to CCR laboratories.

The scientific staff encompasses expertise in a broad range of the basic science disciplines, and a strong collaborative tradition exists between the research staff and their CCR colleagues. The unifying feature of the studies pursued by the investigators is their direct or potential relevance to the overall goal of gaining knowledge and developing cutting-edge tools that can be applied to human diseases. The progress of the PIs and their scientific efforts are monitored through CCR site visits. BSP's contributions focus on cancer and retrovirology, and its researchers seek to understand basic biology, the cellular mechanisms that contribute to carcinogenesis, and the genetic factors that influence disease susceptibility and progression.

Laboratory Animal Sciences Program Directorate

Stephen Jones, Ph.D., Director

The Laboratory Animal Sciences Program (LASP) is a comprehensive resource for National Cancer Institute (NCI) scientists performing animal-based research at the Frederick and Bethesda campuses. The program provides the highest-possible quality of animal care, and assists NCI investigators in the use of healthy animals appropriate for their research objectives. In addition, LASP ensures that all animals are housed, handled, and cared for in a humane manner in accordance with regulatory guidelines; and provides robust scientific support for investigators performing animal-based research.

To support of the diverse research requirements of the scientific community, LASP provides oversight of the Animal Research Facilities, which includes the Laboratory Animal Medicine (LAM), the Animal Health Diagnostic Laboratory (AHDL), and Receiving and Quarantine (R&Q). Through these facilities, LASP guards against the accidental introduction of pathogens in experiments and provides accurate clinical diagnosis and preoperative care. LASP also offers quality animal holding facilities and related services, including the quarantine of imported animals, rederivation of pathogen-carrying strains, and the comprehensive monitoring of the health status of animal research colonies.

In addition to management of Animal Research Facilities, LASP also provides Scientific Support Programs (Cores) to NCI Investigators. LASP Core Support Programs include:

- Mouse Modeling Core (MMC): facilitates the production of genetically engineered mouse models (GEMM) via pronuclear microinjection and gene targeting in embryonic stem cells. MMC also provides a Cryopreservation Laboratory to freeze and archive unique and valuable GEMM strains, thereby guarding against their loss due to disease outbreak or other genetic or environmental factors.
- Pathology/Histotechnology Laboratory (PHL): a comprehensive veterinary pathology and molecular histopathology service that focuses on the phenotypic characterization of animal disease models. PHL capabilities include immuno-histochemistry (IHC), laser-capture microdissection, blood chemistry analysis, and digital whole-slide image capture and analysis.
- Small Animal Imaging Program (SAIP): offers state-of-the-art multimodality animal imaging facility (magnetic resonance imaging [MRI], positron emission tomography/computed tomography [PET/CT], single-photon emission computed tomography/computed tomography [SPECT/CT], ultrasound, X-ray, and optical imaging) for real-time in vivo monitoring of tumor cells and metastases, tracking of gene expression, and assessment of effects of pharmacological interventions.
- Animal Molecular Diagnostics Laboratory (AMDL): employs molecular-based technologies for the detection of animal pathogens, the assessment of the genetic purity of animal-related reagents, and the genotyping of complex genetically engineered mouse strains.
- High-Throughput Animal Genotyping Laboratory (HTAGL): provides a platform for large-scale genetic monitoring and management of complex, genetically engineered mouse model colonies.
- Molecular Imaging Laboratory (MIL): develops and implements new methods for preclinical and clinical in vivo imaging in support of the Molecular Imaging Program, CCR, in Bethesda.
- Gnotobiotics Program: provides researchers with the ability to generate and characterize mice maintained in axenic (germ-free) or gnotophoric (single agent) conditions to better assess the role of the environment or infections on GEMM phenotypes.
- Animal Research Technology: this program assists investigators in undertaking complex procedures and experiments in rodents by providing expertise in the development and implementation of specialized animal research protocols.
- NCI Mouse Repository: a central resource for maintenance and propagation of mouse cancer models and distribution of strains throughout the scientific community (academic, nonprofit, and commercial).

LASP provides management oversight and technical expertise to the Center for Advanced Preclinical Research (CAPR), a program initiative funded by the Center for Cancer Research (CCR) that focuses on the generation of novel animal models of human cancer, and the use of established GEMMs in the development and testing of targeted and effective cancer diagnostics and therapies.

Data Science and Information Technology Program

Anastasia Christianson, Ph.D., Director

The Data Science and Information Technology Program's primary focus is to provide leading-edge data science and information technology skills, tools, and capabilities to accelerate translation of biomedical data to scientific discoveries, medical treatments, and diagnostic and prevention tools for cancer and AIDS patients.

ADVANCED BIOMEDICAL COMPUTING CENTER

Jack Collins, Ph.D., Director

The primary focus of the Advanced Biomedical Computing Center (ABCC) is to support scientific research at NCI at Frederick, NCI-Bethesda, NIH, and other federal agencies through the Economy Act. The ABCC provides bioinformatics, systems biology, data integration and analysis, mathematical simulation and modeling, image analysis and visualization, nanoinformatics, proteomic analysis expertise, and web-enabled application delivery to these communities.

IT OPERATIONS GROUP

Greg Warth, Director

The IT Operations Group (ITOG) is responsible for computational servers, storage servers, virtual machine infrastructure, and the FNLCR network. ITOG focuses on implementing enterprise IT best practices in the areas of computational services, storage, backup, and archiving; batch and application support; server consolidation and virtualization; network infrastructure; unification of voice, teleconferencing, and video communication technologies; and improved infrastructure for colocation of dedicated servers.

CBIIT TECHNICAL OPERATIONS SUPPORT GROUP

Braulio Cabral, Program Director

The Center for Biomedical Informatics and Information Technology (CBIIT) Technical Operations Support consists of several major categories of work related to the development and/or acquisition of biomedical informatics and other information technology resources. CBIIT seeks direct support from NCI at Frederick for resource acquisition and subcontracting, project management and oversight, deliverable review, intellectual property and licensing negotiation, financial management, and coordination with other CBIIT and NCI programs.

CBIIT Technical Operations Support provides these core capabilities as the base of this task order. These activities include the following:

- Support for the definition of scope, time, and budget for information technology activities.
- Development of software products.
- Development of other information technology products, including vocabularies/ontologies, common data elements, data standards, and data or other biomedical capabilities associated with information technology systems.
- Support for open-source resource development activities not directly funded by NCI or funded via non-contract mechanisms.
- Acquisition of commercial information technology products.
- Review and assessment of information technology resources from all subcontract activities and from such non-contract activities as designated by the contracting officer's technical representative.
- Coordination and participation in the National Cancer Informatics Program (NCIP).

HIGH-PERFORMANCE COMPUTING INITIATIVE

Eric Stahlberg, Ph.D., Director

The primary focus of this group is to work collaboratively across the NCI CBIIT and FNLCR to develop a strategy for expanded use of high-performance computing to support cancer research.

INFORMATION SECURITY AND COMPLIANCE OFFICE

Natasha Freeman, Manager

The Information Security and Compliance Office (ISCO) provides IT security auditing, engineering, and incident response support for NCI at Frederick and FNLCR. ISCO supports the life cycle of information security for the scientific mission and administrative functions of NCI at Frederick/FNLCR to ensure the availability of information systems, protect the integrity of information, and protect the confidentiality of intellectual property and patient data.

PROGRAM ADMINISTRATION AND OPERATIONS OFFICE

Megan Kaminski, Director

The Program Administration and Operations (PAO) Office provides support to the Data Science and Information Technology Program (DSITP) to ensure sound business operations by managing financials, contractual compliance, procurement, and other operational functions. The group also facilitates and executes technical project management to support the complex portfolio of projects within the DSITP.

STRATEGIC PROGRAMS

Braulio Cabral, Director

The Strategic Programs group supports exploratory programs focused on the development and integration of advanced technologies and transdisciplinary approaches, infrastructures, and standards to accelerate the creation of publicly available, broadly accessible, multidimensional data sets to benefit the cancer research community.

CLINICAL GROUP

Barry L. Gause, M.D., Director

Clinical Research Directorate

Barry L. Gause, M.D., Director

The Clinical Research Directorate (CRD) was established in November 2006 by bringing together the Clinical Monitoring Research Program (CMRP), and the Quality Assurance programs of the Pilot Plant and the Biopharmaceutical Development Program (QA-BDP). The major purpose for establishing a new directorate was to bring those programs at the clinical end of the translational spectrum under an umbrella that fosters interactions in areas of overlap and provides clinical supervision of such activities. In addition, assigning the QA programs to this directorate was necessary to provide the required autonomy and transparency required of GMP quality assurance operations. Subsequently, three programs have been added to CRD: the Molecular Characterization Laboratory (MoCha), the Biospecimen Research Group (BRG), and the group supporting Therapeutics for Rare and Neglected Diseases (TRND)/ National Center for Advancing Translational Sciences (NCATS).

The objectives of the directorate are (1) to provide clinical research support for clinical trials, (2) provide quality assurance for the production of vaccines and biological agents at the National Institutes of Health (NIH), (3) develop standardized approaches to the acquisition of tumor samples, (4) manage the collection and analysis of tumor and normal tissue on a molecular level, (5) further the field of precision medicine by using next-generation sequencing to direct targeted agents in the treatment of malignancy, and (6) support the development of agents for the treatment of rare and neglected diseases through the management of various projects. CRD provides support for clinical trials management, regulation, pharmacovigilance, and protocol development and navigation, as well as operational support for clinical research. The directorate also provides comprehensive, dedicated clinical research support to major clinical programs within NIH.

In addition, the directorate establishes quality systems at the Vaccine Clinical Materials Pilot Plant (VCMP) and the Biopharmaceutical Development Program (BDP) before the initiation of manufacturing, and follows through on all regulatory aspects of production, including providing support for Investigational New Drugs (INDs). Detailed descriptions of QA activities are presented under the sections for VCMP and BDP.

CLINICAL MONITORING RESEARCH PROGRAM

The primary mission of the Clinical Monitoring Research Program (CMRP) is to provide comprehensive, dedicated clinical research support to major programs within NIH, including the National Cancer Institute (NCI), National Institute of Allergy and Infectious Diseases (NIAID), National Heart, Lung and Blood Institute (NHLBI), National Institute for Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Center for Advancing Translational Sciences (NCATS), National Institute of Mental Health (NIMH), National Institute of Neurological Disorders and Stroke (NINDS), and the NIH Clinical Center. To support the diverse research requirements of the clinical research community, CMRP provides an integrated range of quality services that are functionally organized within CRD. CMRP represents a comprehensive resource for a number of the intramural clinical research programs at NIH. CMRP staff support an extensive variety of high-profile NCI and NIAID initiatives, as described in this report.

As a program, CMRP provides high-quality clinical research support services to meet the expanding and emerging challenges faced by NIH researchers. CMRP recognizes the numerous barriers to conducting clinical research both domestically and in the international setting. Successful completion of our mission directly benefits the mission of NCI, NIAID, and other institutes, and has contributed to improving the overall standards of public health globally. The repertoire of support services provided to clinical researchers throughout the world has expanded dramatically over the last 14 years, assisting researchers in providing the highest-quality clinical research that is compliant with applicable regulations and guidelines, and maintaining data integrity, with the overall goal of protecting human subjects. CMRP continues to support the goal of increasing the capability of international locations to participate and partner in clinical research, and has assisted in the critical development of clinical trial networks across the world.

As the largest program under CRD, CMRP continues to provide regulatory, clinical trials management, pharmacovigilance, protocol development and navigation, and project/program management services to support more than 400 domestic and international studies related to cancer; avian flu/severe human influenza; HIV; HCV; TB; malaria; Ebola; heart, lung, and blood diseases and conditions; parasitic diseases; rheumatic and inflammatory diseases; arthritis; musculoskeletal and skin diseases; and neurological diseases.

Applied and Developmental Research Directorate

Michael W. Baseler, Ph.D., Director

The Applied and Developmental Research Directorate (ADRD) consists of two main program areas: the Clinical Services Program (CSP) and support to the NCI Division of Cancer Treatment and Diagnosis (DCTD). In FY2014, CSP laboratories supported over 150 NCI and National Institute of Allergy and Infectious Diseases (NIAID) clinical trials, as well as trials from additional institutes. Clinical trial support included processing and cryopreserving clinical materials; database tracking of clinical samples received; performing sequential studies of immune function in patients with cancer, AIDS, other infectious diseases, chronic granulomatous disease, and other diseases associated with immune deficiency or autoimmunity; testing viral burden; identifying viral quasi-species; and determining viral mutations associated with drug resistance. These efforts include the evaluation of new technologies and the development of new assays that can be used to monitor patients during therapy. Seven program laboratories performed high-complexity testing under the auspices of CLIA, with test results used to aid in patient diagnosis or treatment decisions.

Laboratories within CSP provide dedicated support to the clinical trials programs at NCI, including the Division of Cancer Epidemiology and Genetics, the Center for Cancer Research, the Division of Cancer Prevention, and NIAID. Support also extends to preclinical and translational research. Several program laboratories provide shared services that can be accessed by other institutes within NIH through the NCI at Frederick Core Service Accessioning System (CSAS). CSP laboratories have also provided support to other government agencies through the Economy Act. CSP also provides technical project management support to the Fisher BioServices Biorepository subcontract.

Biopharmaceutical Development Program Directorate

George Mitra, Ph.D., Technical Director/Program Director

The Biopharmaceutical Development Program (BDP), formerly the Monoclonal Antibody Recombinant Production Program, was established in 1993 to provide dedicated services to the Biological Resources Branch (BRB), Developmental Therapeutics Program (DTP), and Division of Cancer Treatment and Diagnosis (DCTD),

National Cancer Institute (NCI), as well as to provide support to intramural and extramural National Institutes of Health (NIH) investigators, government agencies, and independent parties through interagency agreements or Cooperative Research and Development Agreements (CRADAs). BDP continues to take on new challenges in support of BRB, DTP, and DCTD, NCI.

BDP provides leading-edge development of monoclonal antibodies, recombinant proteins, peptide and DNA vaccines, virus vaccines and oncolytic viruses, gene therapy products, and other biological and immunomodulating agents. BDP maintains biopharmaceutical production and testing facilities that are compliant with relevant current Good Manufacturing Practices (cGMP). BDP provides complete support, from manufacturing feasibility through process development and clinical manufacturing, with all required regulatory documentation. With a staff of 33 highly trained and experienced personnel, BDP's facilities are designed to be flexible, which enables staff to work on multiple projects for a variety of different therapies. BDP concentrates on products that are in early development, beginning with the demonstration of product feasibility on the bench through the production and biomolecular characterization of Phase I/II clinical supplies.

During this period, TA-CIN and Ganitumab were in GMP operations. Significant efforts were expended towards process development of RLIP-76, PTEN-Long, and EBV (gp350) Ferritin.

Vaccine Clinical Materials Program Directorate

David Lindsay, Ph.D., Director

The VCMP supports the Vaccine Research Center at NIAID/NIH to advance pre-clinical and clinical research and development activities by producing clinical-stage vaccine candidates for infectious diseases of global significance. Key activities include procurement services for research/development, technology transfer of manufacturing processes and analytical methods, scientific subcontracting, pilot scale GMP production of drug substance and vialed drug product at the Frederick pilot plant and or subcontract locations, product release testing and stability testing, regulatory/IND support, and inventory/supply of a range of vialed drug products, including placebo and adjuvants to clinical sites. The directorate is presently engaged in multiple programs including HIV-AIDS broadly neutralizing monoclonal antibodies, influenza-flu nanoparticles, respiratory syncytial virus recombinant vaccine development, malaria and tuberculosis vaccine development, as well as emerging and re-emerging diseases including flaviviruses like Zika.

VCMP has a total of 136 employees. Most of these employees have completed at least a bachelor's degree and have significant experience in bio/pharmaceutical GMP operations.

OPERATIONS AND FINANCIAL GROUP

Kathy Terlesky, Ph.D., Chief Operating Officer

Contracts and Acquisitions Directorate

Bob Mason, Director

The Contracts and Acquisitions Directorate (C&A) provides contracts, export, intellectual property, strategic agreement, acquisition (purchasing/subcontracting), receiving, warehousing, distribution, and transportation support for the Frederick National Laboratory for Cancer Research (FNLCR) and NCI at Frederick operations.

During the year, continued improvements to the organizational structure, staffing, training, and other performance enhancements previously briefed to NCI Management Operations and Support Branch (MOSB) and Office of Scientific Operations (OSO) leadership in July of 2015 continue. These enhancements were again briefed in March 2016 with an additional update due in October 2016. Enhancements implemented include but are not limited to:

- Installation of senior leadership and management for the Acquisitions Department leading to focused performance improvements department-wide.
- Dedicated support for all intellectual property and strategic agreement activities.
- Additional staff and expertise within the Contracts Department allowing for greater capabilities and capacities related to task order management and export compliance.

- Enhanced communication protocols to support a focused interaction and issue resolution process with MOSB.
- Increased use of technology enhancements within the logistics organization.
- Directorate-wide establishment of performance metrics to assist management in ensuring quality support is delivered on an on-going basis.
- Increased transparency and communications with customers as related to support activities within all directorate departments.
- Implementation of internal survey tools to gather information regarding levels of support and education that may be needed. External implementation planned for later this year.
- Establishment of a directorate-wide training program leading to increased compliance and performance in all areas (on-going).
- Implementation of heightened processes associated with "personal services" mitigation and Consultant Agreement oversight (on-going).

Management Support Directorate

Richard A. Pendleton, MBA, Director

The Management Support Directorate (MSD) is responsible for providing solutions to administrative, operational, and program-specific activities for the Operations and Technical Support (OTS) and indefinite delivery/indefinite quantity (IDIQ) contracts. This involves planning and implementing integrated policies and procedures and providing direct program and surge support in response to Leidos Biomed directorate's requirements. MSD staff also work with NCI program staff on programs and projects that support the NCI at Frederick community. A major emphasis for MSD is management of quality assurance and customer satisfaction systems to assess project status and facilitate the initiation of working project teams. MSD staff members support a wide range of activities related to OTS contract administration and operational support as identified by senior management.

Human Resources Directorate

Christopher March, Director

The Human Resources Directorate (HR) works in partnership with managers and their teams to support the mission of Leidos Biomed staff as they work on behalf of the Frederick National Laboratory for Cancer Research (FNLCR).

HR provides leadership and guidance in the development, implementation, and equitable administration of policies and procedures, thus fostering a positive work environment. The core services and competencies include talent acquisition, organizational and employee development, compensation and benefits, HR information management, employee relations, and counseling and regulatory compliance.

Facilities Maintenance and Engineering Directorate

Dante Tedaldi, Ph.D., P.E., Director

The Facilities Maintenance and Engineering Directorate (FME) is a full-service operation, providing in-house design, construction management, maintenance, and facilities management functions. FME plans, designs, develops, and executes facility improvements for the Frederick National Laboratory for Cancer Research (FNLCR). An emphasis on interactive working relationships with scientists, directorate staff, and administrative management results in cost-effective solutions in response to the changing needs of the researchers. FME remains committed to serving the evolving needs at FNL.

FME provides ongoing support to the scientific mission with routine daily operations, maintenance duties, and around-the-clock emergency maintenance/repair services. FME personnel continually strive to maintain and improve the physical facilities and environment for all who work at FNL.

Environment, Health, and Safety Directorate

Terri S. Bray, Director

The Environment, Health, and Safety Directorate (EHS) is dedicated to ensuring a safe, healthful, and environmentally friendly workplace for all employees of, and visitors to, NCI at Frederick.

It is the policy of NCI at Frederick to create and maintain a healthy and safe workplace, and to promote a healthy workforce as its most valuable and enduring resource. EHS provides comprehensive health services to NCI at Frederick employees, as well as emergency medical services and treatment for accidental injury or illness sustained by NCI at Frederick employees.

Our goal is to maintain and develop safety programs and regulations that establish guidelines for full compliance with all applicable federal, state, and local occupational safety and environmental laws and regulations.

Financial Operations Directorate

Tim Boyle, Director

The Financial Operations Directorate oversees all finance-related activities in support of the Frederick National Laboratory for Cancer Research FFRDC, including the following accounting, treasury and finance functions: financial planning and analysis; general accounting; payroll; accounts payable; accounts receivable and billing; business information systems; and internal audit and co-compliance. The directorate's mission is to administer an enterprise-level, integrated, financial management program to support the full mission of the FNLCR; establish fiscal policies that ensure accountability for, and control of, government funds; provide timely, relevant, and clear financial analysis and reporting to assist in managing fiscal resources; and deliver accurate, timely and complete financial information to FNLCR stakeholders.

Appendix B: Publications

leidos

Leidos Biomedical Research, Inc.

Leidos Biomedical Research, Inc.

2015-2016 Annual Report

Appendix B

Publications

OFFICE OF THE PRESIDENT

Cancer Genomics Research Laboratory

JOURNAL ARTICLES

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ABSTRACTS

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Dobrovolskaia MA: Pre-clinical immunotoxicity studies of nanotechnology-formulated drugs: Challenges, considerations and strategy. 7th International Conference on Drug Discovery and Therapy. Sharjah, Dubai, UAE.

AIDS and Cancer Virus Program Directorate

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Applied and Developmental Research Directorate

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