

NATIONAL CANCER INSTITUTE AT FREDERICK (NCI@F)
INSTITUTIONAL BIOSAFETY COMMITTEE
MINUTES
MARCH 22, 2016

CALL TO ORDER / ANNOUNCEMENTS

The NCI at Frederick Institutional Biosafety Committee was convened at 12:00 pm in Building 426 Conference Room with the following members in attendance:

Voting (Quorum = 8)

- | | |
|---|---|
| <input type="checkbox"/> Michael Baseler (regrets) | <input checked="" type="checkbox"/> Sarah Hooper |
| <input checked="" type="checkbox"/> Theresa Bell | <input checked="" type="checkbox"/> Serguei Kozlov |
| <input checked="" type="checkbox"/> Rev. David Betzner | <input checked="" type="checkbox"/> Dan McVicar |
| <input checked="" type="checkbox"/> Stephen Creekmore | <input checked="" type="checkbox"/> Bradley St. Croix |
| <input checked="" type="checkbox"/> Eric Freed | <input checked="" type="checkbox"/> Lucien Winegar |
| <input type="checkbox"/> Melinda Hollingshead (regrets) | <input type="checkbox"/> Sharon Altmann (regrets) |
| <input checked="" type="checkbox"/> Stephen Hughes | <input checked="" type="checkbox"/> Robin Sun |
| <input checked="" type="checkbox"/> Antonio Valentin | <input checked="" type="checkbox"/> Jatinder Gulani |

Non-Voting

- Walter Hubert
- Karen Barber

Visitors

- Ted Witte
- Sam Denny
- Sheryl Ruppert
- Beverly Keseling

APPROVAL OF MINUTES FROM THE FEBRUARY 2016 MEETING

The minutes from the February 16, 2016 meeting were approved. A motion to approve and a second were made. (For: 12; Against: 0; Abstain: 1)

ACCIDENT REVIEWS A lab tech was wiping forceps with disinfectant when the forceps pierced his finger. It was not determined if the forceps were disinfected enough to inactivate any potential infectious human material. The employee was put on prophylaxis until the screening of the material was completed. All sharps with fine points have been removed from this lab.

REVIEW OF PROTOCOLS

NEW REGISTRATIONS

- ❖ Giorgio Trinchieri – 16-09: Effects of local and systemic microbiome on immune responses. The focus of this work is to complement the goals of the Cancer and Inflammation program by investigating the role of the microbiome on the development of the immune response to cancer. The microbiome refers to the bacteria that live in or on the body. These bacteria are known to play

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a major role in the way the immune system develops and responds to challenges like germs or cancer. Most of the work on the microbiome has been performed on the bacteria in the intestine, and other bacterial populations (for example in the skin, urogenital tract, or lungs) have not been as well studied. We hope to focus our efforts on studying the microbiome of both the intestinal tract and the lungs to see what its effects are on the immune response of the lungs to cancer metastasis. By studying the effects of the microbiome on immune functioning, we hope to gain a better understanding of how we might modulate the bacteria in the body to help treat or prevent disease.

It is very critical and important to understand the development and progression of lung cancer. There are not many mouse models available for lung cancer. We are going to use a p53/ K-ras LSL mice, (developed by Dr. Perwez Hussain, NCI, NIH), which is a conditional genetic mouse model, and a specific activation of cancer can be achieved by utilizing an Ad-cre virus. This virus would be introduced to the lung through intranasal or intra tracheal instillation by a non-invasive method. That would all us to induced primary cancer only in the lung but not any other place. This is a powerful technique to investigate localized effect of lung cancer and the interaction of cancerous cells with the healthy immune cells of the mice. This protocol would require 1-5 minutes per mouse with an additional 45 minutes to complete the entire experiment including the recovery of the mice from anesthesia. Lung cancer (metastasis) can be studied within 2-3 weeks after the administration of the ad-cre virus. Ultimately this conditional mouse model would help us to test novel chemopreventatives and to develop strategies for anti-cancer therapeutics. A motion to approve pending clarifications was made by S. Kozlov and seconded by S. Creekmore. (For: 13; Against: 0; Abstain: 1)

- ❖ Beverly Keseling – 16-11: Attenuated recombinant Respiratory Syncytial Virus Vaccine RSV 275, technology transfer and GMP clinical production. The Respiratory Syncytial Virus (RSV) is a virus belonging to the Pneumovirus genus. It is an enveloped spherical virus from 100 to 350 nm in diameter. The virion consists of eight structural proteins. The genome consists of a linear, single-stranded negative sense RNA (~ 15.2 kb). RSV primarily infects human epithelial cells in the nasopharynx. RSV occurs worldwide and is the most common cause of bronchiolitis and pneumonia in infants and young children. Most children will be infected by age two to three and there is no vaccine available. Several live-attenuated RSV vaccines have been evaluated in clinical trials in adult and pediatric populations as part of NIAID's ongoing RSV vaccine development program. All of these trials have been published in peer-reviewed journals. Three new RSV vaccines designated as RSV MEDI delta M2-2, LID delta M2-2 and RSV 275 are either in phase I trials or will be on trial in 2016 and 2017 (see attached Dr. Buchholz's Power Point slides 7 and 8). Information is supplied for RSV MEDI delta M2-3 and LID delta M2-2 because these were developed before RSV 275.

The Laboratory of Infectious Diseases (LID), National Institute of Allergy and Infectious Diseases (NIAID), Johns Hopkins and Medimmune have collaborated to develop a candidate attenuated RSV vaccine by the synthesis of DNA from an RNA template, via reverse transcription, producing complementary DNA (cDNA). This allows recovery of infectious virus entirely from cDNA in qualified cells under defined conditions. The method provides virus that is free of adventitious

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agents. This method introduces predetermined mutations into infectious RSV. By deleting the M2-2 gene the virus was shown not to cause disease but produces high levels of viral antigens. Additional attenuating mutations were introduced into the LID delta M2-2 backbone to create vaccine candidates that would be more restricted in replication than LID delta M2-2. The RSV275 vaccine candidate is closely related to the LID delta M2-2. It was generated by reverse genetics, and contains two attenuating elements, namely the M2-2 deletion, and a set of 5 amino acid substitutions in the N, F, and L proteins. RSV containing the M2-2 deletion was highly restricted in replication in three animal models: mice, chimpanzees, and African green monkeys. A motion to approve pending clarifications was made by T. Bell Toms and seconded by R. Sun. (For: 12; Against: 0; Abstain: 1)

Javed Khan – 15-61: Molecular targeting of rhabdomyosarcoma with combinations of small molecule drugs. Rhabdomyosarcoma (RMS) accounts for 3% of all childhood cancers, constituting approximately 350 new cases annually in the US. This tumor, which is derived from skeletal muscle progenitors, is sub-divided into two major genetic and histologic subtypes: embryonal (ERMS, PAX3-FOXO1 fusion negative) and alveolar (ARMS, PAX3-FOXO1 fusion positive). ERMS has a better prognosis than ARMS, with relapse-free survival rates approaching 70- 80% for ERMS patients with localized disease. However, the 5-year survival rate for patients with metastatic RMS at diagnosis or relapsed disease is poor irrespective of the subtype: 25% for ERMS and 10% for ARMS. Addition of targeted agents are needed to improve overall survival in relapsed or refractory RMS. We have identified several novel drug combinations that are effective in RMS cell killing in culture. In the planned experiments, we will test the efficacy of these drug combinations in mouse models of RMS. A motion to defer this registration to the March Committee meeting, for further clarification, was made by J. Gulani and seconded by S. Altmann. (For: 13; Against: 0; Abstain: 0)

RENEWAL REGISTRATIONS

- ❖ Jayanta Sinha – 16-05(12-59): Plasmid DNA vaccine production. The purpose of this registration renewal is to describe a manufacturing platform for pDNA vaccines. The scale of manufacturing ranges from 200mL starter cultures up to the 100L scale for manufacture of materials for human clinical trials.

The specific processes used for manufacture of plasmid DNA based vaccines differs from product to product, but can typically be divided into five stages. In the first stage, the bacterial cell is transformed with the plasmid DNA to be produced. Once an acceptable transformant which expresses the plasmid is selected, it is grown in small-scale shake flask cultures to provide an adequate amount of cells for cell banking activities. Cell banking involves storing a large volume of cells in small aliquots to provide a uniform suspension for all future experiments. Cell banking is typically only performed once or twice per plasmid. Stages 2-5 of the process are performed for each lot of vaccine produced. In stage 2, upstream processing, a specific volume of the banked cells, are inoculated into a fermentor containing a growth medium, and the cells are grown under

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controlled conditions to produce the plasmid DNA product. Since the product remains in the cell, rather than being secreted into the medium, the cells are subsequently harvested, using a filtration apparatus, to separate them from the culture medium in which they were grown. In stage 3, lysis and clarification, buffer solutions are added to the cells to chemically disrupt the membranes and free the plasmid DNA. At this stage, the product consists of about 96% cellular debris (proteins, genomic DNA, cell membranes) and about 4% plasmid DNA, the product of interest. Thus, additional processing is required to provide a vaccine of the high purity required for a biopharmaceutical product. This suspension is filtered and concentrated to remove some of the cell debris and sent to the fourth stage, downstream processing. In stage 4, various unit operations including column chromatography and filtration are used to produce a product which meets the pre-determined quality specifications in place for DNA vaccines. In stage 5, highly purified plasmid, which has been formulated into the buffers and concentrations used in the final vaccine product is filled into vials. The final formulated vaccine is stored under the appropriate conditions until all subsequent product testing is completed and the FDA has approved the product for investigational use in human clinical trials.

All of the activities in stages 1-5, described above, will be conducted at the Vaccine Pilot Plant which operates under U.S. Good Manufacturing Practices (GMPs) which are regulations drafted and enforced by the Food and Drug Administration (FDA).

In addition to manufacturing the vaccine product, the VPP contains Quality Control labs to ensure that the product meets pre-determined specifications. Prior to using the vaccine in humans, rigorous release testing is required to ensure that the vaccine meets the pre-determined specifications for purity, sterility, identity and contaminant profiles. One test used for the release of the vaccine involves transfecting the plasmid into a human cell line and confirming, using various analytical techniques, that the Ebola glycoprotein is expressed. These studies will be conducted at small scales (less than 100mL) in areas of the facility separate from those used to manufacture the vaccine and with appropriate engineering controls. A motion to conditionally approve this registration with clarifications was made by S. Hughes and seconded by B. St. Croix. (For: 13; Against: 0; Abstain: 0)

Peter Johnson 16-03 (11-37): Molecular genetics of the C/EBP family of transcription factors. Our laboratory is interested in the regulatory pathways that distinguish normal cells from cancerous cells, focusing on the control of gene expression by specific DNA-binding transcription factors. In particular, our research involves the C/EBP (CCAAT/enhancer binding protein) family of transcription factors. We aim to understand the regulation of C/EBPs by oncogenic signals as well as their target genes that contribute to tumorigenesis or tumor suppression. We employ genetic approaches involving genetically engineered mice (GEM) and cells derived from these animals, cell biological studies in tissue culture, and biochemical experiments using mammalian cell extracts or recombinant proteins produced in *E. coli*. Experiments using mammalian cells in culture involve transient or stable introduction of exogenous vectors expressing normal or mutant genes of interest and determining their effects on cell proliferation, oncogenic transformation, and activation of target genes. We also use transfected cells to conduct protein activity assays and to assess protein: protein or protein: DNA interactions, as well as to ablate expression of specific genes using RNA interference approaches. Experiments in mice will use GEM animals as detailed in

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my short registration form for breeding transgenic and KO mice. A motion to defer this registration to the April Committee meeting, for further clarification, was made by D. Betzner and seconded by S. Hughes. The Committee voted to notify the Office of the Director regarding the challenges concerning this IBC registration renewal. The Committee requested that the laboratory program not be permitted to perform work with pBABE (retroviral vectors) when used in combination with hot oncogenic RAS both in vitro and in vivo, until they appropriately acknowledged the hazards and assure the Committee that the hazards and their mitigation measures have been identified and communicated to the staff performing the work. (For: 13; Against: 0; Abstain: 0)

Alan Weissman – 16-06 (10-13): Tissue culture and animal models for the Ubiquitin system. In our efforts to study ways of controlling oncogenic transcription factors with designed molecules, we are carrying out a pilot study aimed at modulating the function of the bacterial transcription factor sigma 54. Sigma 54 is responsible for the pathogenic properties of several microorganisms. The experiments revolve around compound design and activity in bacterial cells using E.coli as our model organism. A motion to conditionally approve pending clarifications was made by S. Kozlov and seconded by J. Gulani. (For: 13; Against: 0; Abstain: 0)

Doug Kuhns – 16-07 (12-56): Laboratory Operations of the Neutrophil Monitoring Lab. The Neutrophil Monitoring Laboratory (NMI), Applied/Developmental Research Directorate, provides clinical trials support to NIAID. The primary function of NML is to receive, process, and perform functional assays on clinical specimens from patients with chronic granulomatous disease, mycobacterial infections, Job's syndrome or other primary immunodeficiency diseases. The results of this work aid in the diagnosis and severity of the disease. The NML also monitors the percent of cells that produce normal levels of reactive oxygen species from transplant patient. The human clinical specimens received by NML are collected under NIAID or NCI Institutional Review Board(IRB)-approved clinical protocols. Some clinical specimens are also obtained under IRB-approved protocols conducted at other domestic and international sites. A motion to conditionally approve pending clarifications was made by E. Freed and seconded by L. Winegar. (For: 13; Against: 0; Abstain: 0)

Dan McVicar/Jonathan Weiss – 16-10(13-50): Maintenance and production of mutant mouse strains for the study of the pathogenesis and therapy of cancer. Breeding only.

OUTSTANDING ITEMS

- ❖ Andre Nussenzweig – 15-60 (12-63): Expression of MLL fusion proteins in BM cells for transplantation to assess tumor formation in vivo. Our lab is studying the role of DNA repair response genes in tumorigenesis. We believe that deletion or inhibition of proteins in the DNA repair response pathways can impair or reverse MLL fusion induced acute myeloid leukemia (AML). To test this in vivo we will 1) transfect HEK293T cells to produce murine ecotropic retrovirus carrying DNA encoding a murine MLL fusion protein, 2) infect wildtype or knockout mice BM cells

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to stably express the MLL fusion protein, 3) IV inject these transformed cells into recipient mice, either C57BL6 or NRG, 4) follow progression of leukemia to up to 140 days. A motion was made to defer to March 2016 with the request to resubmit the registration. (For: 11; Against: 0; Abstain: 0)

Vinay Pathak – 15-62 (07-37): Mechanisms of retroviral replication. We seek to understand how retroviruses replicate and how they evolve to acquire resistance to antiviral drugs and human antiviral defense proteins called restriction factors. We are studying how APOBEC3 proteins, a family of host cytidine deaminases, inhibit HIV-1 replication, and how the viral Vif protein overcomes these host defenses. We are screening for small molecule inhibitors of APOBEC3-Vif interactions, elucidating the structure and function of APOBEC3 proteins, and identifying host genes that facilitate the Vif-APOBEC3 interactions. We are exploring mechanisms of antiviral drug resistance and retroviral replication. We are also using APOBEC3 proteins tagged with fluorescent proteins such as yellow fluorescent protein to label HIV-1 virions that can complete one cycle of replication but have defects in many essential genes such as envelope and/or reverse transcriptase, and accessory genes such as Vif, Vpr, Vpu and Nef. A motion to conditionally approve pending clarifications was made by A. Valentin and seconded by S. Creekmore. (For: 13; Against: 0; Abstain: 0)

AMENDMENTS

Twenty-nine amendments were processed and approved between January and February IBC meetings.

OTHER BUSINESS

ADJOURNMENT

The meeting adjourned at 2:35 pm.

Next meetings: April 19, 2016 May 17, 2016