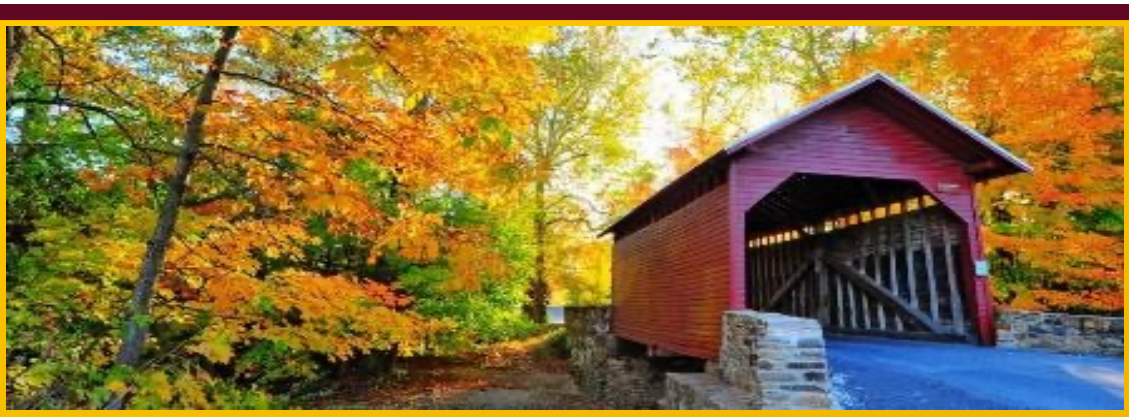


CEHCV
Fall Think Tank 2023

Sponsored by The Center of Excellence in HIV/AIDS and Cancer Virology, CCR, NCI

Building 45 Natcher Conference Center
Room E1/E2

Friday December 8, 2023

8:45am - 5:00pm

NATIONAL CANCER INSTITUTE

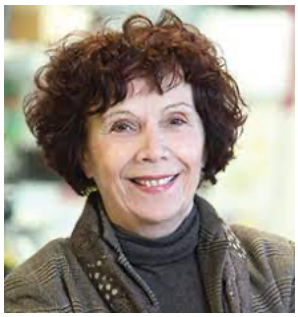
CENTER FOR CANCER RESEARCH

NATIONAL INSTITUTES OF HEALTH

Invited Speakers

John Schiller, Ph.D.
Deputy Chief, Laboratory of Cellular Oncology

The Surprises in Developing HPV Virus-Like Particles as an HPV Prophylactic Vaccine and a Broad-Spectrum Cancer Therapy



Genoveffa Franchini, M.D.
Senior Investigator, Vaccine Branch

Innate and Adaptive Immunity to Prevent SIV/HIV Infection

Ramya Ramaswami, M.B.B.S., M.P.H.
Lasker Clinical Research Scholar

Beneath the surface - Exploring Kaposi sarcoma and other KSHV-associated disorders

Center for Excellence in HIV and Cancer Virology
Fall Think Tank 2023
Natcher Conference Center (Building 45), E1/E2

AGENDA

8:50 – 9:00 ***Opening Remarks –Vinay Pathak and Cynthia Masison***

9:00 – 10:00 **John Schiller-"The Surprises in Developing HPV Virus-Like Particles as an HPV Prophylactic Vaccine and a Broad-Spectrum Cancer Therapy."**

Chairs: Prabha Shrestha and Alice Duchon

10:00 – 10:15 Ameera Mungale-"Vascular Endothelial Growth Factor Receptors Regulate the KSHV Replication Cycle"

10:15 – 10:30 Lulu Yu-"HPV Oncogenes Expressed From Only One of Multiple Integrated HPV DNA Copies Drives Clonal Expansion in Cervical Cancer"

10:30 – 10:45 Subhajit Chatterjee-"Sublethal Doses of Genotoxic Chemotherapeutics and Antivirals Promote Polyomavirus Replication"

10:45 – 11:00 Sarkis Sarkis-Infection by HTLV-1A and HTLV-1A/C Chimeric Virus Results in Distinct Proteomic Inflammatory Profile in Rhesus Macaques"

11:00-– 11:15 Joshua Gluck-"Intact Proviruses Persist in Expressed Genes in People with Non-Suppressible HIV on Long-term ART"

11:15 – 11:25 ***Break……***

Chairs: Debananda Das and Mohammad Arif Rahman

11:25 – 11:40 Yuta Hikichi-"High-level Resistance to Integrase Inhibitors Conferred by Mutations Outside Integrase"

11:40 – 11:55 Ryan Burdick-"HIV-1 Uncoating requires reverse transcription of long double-stranded DNA"

11:55 – 12:10 Xue Zhi Zhao-"Structure-guided Optimization of HIV Integrase Strand Transfer Inhibitors with Improved Efficacy Against a Broad Panel of Viruses Having Resistant Mutant Forms of Integrase"

12:10 – 12:25 Jonathan Kitzrow-"HIV-1 Transcription Start Site Mutations Negatively Impact Selective Genome Packaging"

12:25 – 12:55 ***Lunch Break……***
12:55 – 2:00 ***Poster Session (rooms F1/F2)…… ……..……..***

2:00 **Afternoon session**

2:00 – 3:00 **Genoveffa Franchini-"Innate and Adaptive Immunity to Prevent SIV/HIV Infection "**

Chairs: Abdul Waheed and Yongjun Sui

3:00 – 3:15 Dipanwita Mitra-"Vaccination with Replication-Dead Murine Gammaherpesvirus Protects Against Wild-Type Infection and Reactivation in Mice"

3:15 – 3:30 Annemarie Glassy-"Clonal Expansion and Driving Forces of HIV-1 Persistence in Anatomic Compartments"

3:30 – 3:45 Scott Norberg– "Manufacturing TCR-T cell Therapy Treatments Targeting HPV E7 in Patients with HIV and HPV-associated Cancer"

3:45 – 4:00 Emma Treco-"The Effects of Novel Cereblon-binding Immunomodulators on Primary Effusion Lymphoma"

4:00 – 4:15 Massimilliano Bissa-"In Vivo Treatment with Insulin-like Growth Factor 1 Reduces CCR5 Expression on Vaccine-Induced Activated CD4+ T cells"

4:15-4:25 ***Break……***

4:25 – 4:50 **Ramya Ramaswami-"Beneath the Surface - Exploring Kaposi Sarcoma and Other KSHV-associated Disorders"**

4:50 – 5:00 ***Closing Remarks and Announcement of Travel Award Winners***

Oral Presentations

Vascular endothelial growth factor receptors regulate the KSHV replication cycle.

Ameera Mungale, Sarah Dremel, and Joseph Ziegelbauer, HAMB, CCR, NCI, NIH

Kaposi sarcoma-associated herpesvirus (KSHV) is the etiologic agent of HIV-associated malignancies including Kaposi sarcoma (KS). HIV infection promotes KS development via angiogenic and pro-inflammatory factors. Additionally, KSHV infection drives angiogenesis as observed in highly vascularized KS lesions and in vitro infection, yet the direct mechanism remains unclear. In RNA-Seq data from cancerous skin KS versus normal tissue (n=10 patients) we found the pro-angiogenic vascular endothelial growth factor receptor-3 (VEGFR3/FLT4) significantly upregulated 7-fold $\pm$ 1.2. This induction was more pronounced in cell culture with VEGFR3 increasing 500-fold after lytic reactivation. To probe VEGFR3's role during infection, we performed siRNA depletion in primary lymphatic endothelial cells (LEC) prior to KSHV infection (MOI ~1). VEGFR3 knockdown caused significant increases in aspects of the viral life cycle. We observed increases in viral genome replication (2-fold), viral transcripts (1.5-fold), nascent particles (3-fold), and infectious progeny (30-fold) with no change in viral entry. RNA-sequencing of knockdown samples at a lower MOI showed broad upregulation of all viral genes across transcriptional kinetic class. Pathway analysis of host transcripts showed inhibition of NOS and HIF1a signaling, which are also known to be involved in KSHV infection, suggesting FLT4 may be modulating infection from both host and viral sides. The VEGF family is functionally redundant as VEGFR2/KDR binds common ligands and was also upregulated in KS lesions and cell culture models. VEGFR2 siRNA depletion in a LEC de novo infection model significantly decreased viral genome replication (3-fold) and immediate early and early viral transcripts (up to 5-fold) but did not change entry, nascent particles and infectious progeny. These data require follow-up but suggest VEGFR2 and VEGFR3 have opposing roles in the KSHV life cycle. We propose a model in which VEGFR3 is impeding lytic replication such that KSHV preferentially enters latency where it can more favorably drive pro-angiogenic processes.

Presenter: Ameera Mungale
Position: Graduate Student

HPV oncogenes expressed from only one of multiple integrated HPV DNA copies drive clonal expansion in cervical cancer

Lulu Yu[1], Vladimir Majerciak[1], Alexei Lobanov[2], Sameer Mirza[3], Vimla Band[3], Haibin Liu[1], Maggie Cam[2], Stephen H. Hughes[4], Douglas R. Lowy[5], Zhi-Ming Zheng[1]*

[1]Tumor Virus RNA Biology Section, HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA

[2]CCR Collaborative Bioinformatics Resource (CCBR), National Cancer Institute, Bethesda, MD 20892, USA

[3]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE 68198, USA

[4]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA

[5]Laboratory of Cellular Oncology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892Abstract

The integration of HPV DNA into human chromosomes plays a pivotal role in the onset of papillomavirus-related cancers. Here we provide compelling evidence that, despite the presence of numerous integrated viral DNA copies, virus-host fusion transcripts originate from a single integrated HPV DNA in HPV16 and HPV18 cervical cancers from TCGA samples, a few Chinese samples, and cervical cancer derived cell lines. The host genomic elements neighboring the integrated HPV DNA are critical for the efficient expression of the viral oncogenes that leads to clonal cell expansion. The fusion RNAs that are produced use a host RNA polyadenylation signal downstream of the integration site and almost all involve splicing to host sequences. In cell culture, siRNAs specifically targeting the host component of the virus-host fusion transcripts effectively silenced viral E6 and E7 expression. This, in turn, inhibited cell growth and promoted cell senescence in HPV16+ CaSki and HPV18+ HeLa cells. Showing that HPV E6 and E7 expression from a single integration site is instrumental in clonal cell expansion sheds new light on the mechanisms of HPV-induced carcinogenesis and could be used for development of precision medicine tailored to combat HPV-related malignancies.

Presenter: Lulu Yu
Position: Research fellow

Sublethal doses of genotoxic chemotherapeutics and antivirals promote polyomavirus replication

Subhajit Chatterjee and Gabriel Starrett, LCO, CCR, NCI, NIH

Transplant recipients are thought to be at an increased risk of virus-mediated cancers due to weakened immune control. Some chemotherapeutics, antivirals, and immunosuppressive treatments can damage DNA, and could also affect persistent, subclinical DNA virus infections. Polyomaviruses (PyV) are ubiquitous pathogens that depend on host DNA damage responses for replication and are linked to cancer in immunosuppressed patients. These viruses can integrate into tumor genomes, sustaining oncogene expression. Our starting hypothesis was that DNA-damaging drugs in transplant recipients might promote viral integration, leading to cancer.

To investigate this hypothesis, we conducted experiments using immortalized bladder epithelial cells (HBLAKs) infected with BK polyomavirus (BKPyV) and exposed them to chemotherapeutic agents (Etoposide, Cisplatin, 5-Fluorouracil), immune-conditioning drugs (Cyclophosphamide, Fludarabine, Busulfan) and antiviral drugs (Cidofovir, Ganciclovir, Acyclovir), all known to be genotoxic. Surprisingly, our preliminary findings indicate that under mild genome instability (at $IC10$ concentrations for 48 hours) following infection, BKPyV replication increases four- to five- fold compared to untreated cells. Prolonged exposure for five days further amplifies BKPyV replication, resulting in a nine- to twelve-fold increase over untreated cells. Intriguingly, a six- hour pre-treatment before infection also enhances viral replication by eleven- to fifteen-fold compared to untreated cells, without continued drug exposure. Additionally, we observed increased susceptibility to BKPyV infection following these pre-treatments. Further, we conducted validation experiments under HBLAKs organotypic culture conditions. We are currently investigating alterations in viral genome replication intermediates, and integration rates to decipher the mechanisms underlying these observations. Additionally, we are scrutinizing metagenomic data from skin swabs taken shortly after busulfan and fludarabine treatment in patients to determine if these drugs acutely influence viral replication and recombination prior to the onset of immunosuppressive effects. These findings suggest that even low-level DNA damage resulting from therapeutic interventions can promote PyV replication, contributing to the diseases observed in immunosuppressed patients, including cancer.

Presenter: Subhajit Chatterjee
Position: Postdoctoral Fellow

Infection by HTLV-1A and HTLV-1A/C chimeric virus results in distinct proteomic inflammatory profile in rhesus macaques

Sarkis Sarkis [1], Gutowska Anna [1], Rahman Arif Mohamad [1], Moles Ramona [1], Schifanella Luca [1], Goldfarbmuren Katherine [1], Bissa Massimiliano [1], Doster Melvin [1], Silva de Castro Isabela [1], Omsland Maria [1], Galli Veronica [1], Washington-Parks Robyn [1], Choo-Wosoba Hyoyoung [2], Breed Mattew [3], Kramer Joshua [3], Killoran Kristin [3], Purcell FJ Damian [4], Pise-Masison Cynthia [1,5], Franchini Genoveffa [1]

[1] Animal Models and Retroviral Vaccines Section, Center Cancer Research National Cancer Institute, Bethesda, Maryland, USA; [2] Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA; [3] Laboratory Animal Sciences Program, Leidos Biomedical Research Inc., Frederick National Laboratory, Frederick, Maryland, USA. [4] Viral Infection Diseases Doherty institute, The University of Melbourne, Australia. [5] Office of Scientific Programs, Center Cancer Research, National Cancer Institute, Bethesda, Maryland, USA.

Abstract

HTLV-1C, the most divergent variant, is endemic in indigenous populations of central Australia and Oceania. HTLV-1A and C appear to differ in their clinical manifestation, with HTLV-1C being associated with a more prominent life-threatening lung disease. The highest genetic variability between HTLV-1A and HTLV-1C is at the 3'end of the virus including a lack of a translational initiation codon for *orf-I* and a polymorphism in *orf-II* and *HBZ.* Since the expression of *orf-I* is essential for HTLV-1A fitness and viral persistence *in vivo*, we investigate whether expression of *orf-I* in type C occurs by an alternative mechanism and whether those virus variants cause different inflammatory profiles in rhesus macaques in vivo. We engineered a chimeric molecular clone by inserting into the HTLV-1A$_{WT}$ backbone, the type C *orf-I*, *II*, *III*, *IV* and the 3'LTR, generating the HTLV-1A/C*oI-L* chimeric virus. HTLV-1A/C*oI-L* expresses all viral mRNA, encoding the HTLV-1 structural, regulatory, and auxiliary proteins; with a conservation of the splice acceptor sites located in the pX region. Moreover, HTLV-1A/C*oI-L* expresses a doubly spliced mRNA transcript, that juxtaposes the first exon of *rex* and its ATG in frame to *orf-I*, encoding a cytoplasmic 16Kda protein. In addition to its transmissibility to primary human and macaque $CD4^+$T cells in vitro, seroconversion occur in 75% of animals inoculated with the HTLV-1A/C*oI-L*. Triple depletion of NK, $CD8^+$T and monocytes subsets accelerated the seroconversion and exacerbated viral replication in all animals exposed to this chimeric virus. Furthermore, infection of macaques with HTLV-1A/C*oI-L* and HTLV-1A reveals a distinct proteomic inflammatory profile in plasma that may account for differences in their pathogenicity in humans. An understanding of the HTLV-1C pathogenicity and inflammatory profile in macaques, if paired with equivalent results in humans, will provide a suitable animal model for testing approaches to prevent inflammation and lung manifestation associated with HTLV-1C.

Presenter: Sarkis Sarkis

Position: Postdoctoral Fellow

Intact Proviruses Persist in Expressed Genes in People with Non-Suppressible HIV on Long-term ART

Joshua A. Gluck[1], Sean C. Patro[2], Elias K. Halvas[3], Kevin Joseph[3], Nathan McKenna[3], Shuang Guo[2], Shadab Parvez[1], Jason W. Rausch[1], John M. Coffin[4], Xiaolin Wu[2], John W. Mellors[3], Stephen H. Hughes[1], Mary F. Kearney[1]

[1]National Cancer Institute, Frederick, MD, USA, [2]Leidos Biomedical Research, Inc, Frederick, MD, USA, [3]University of Pittsburgh, Pittsburgh, PA, USA, [4]Tufts University, Boston, MA, USA

The persistence of replication-competent proviruses during ART is a key barrier to an HIV cure. A previous study identified 3 cell clones carrying replication-competent proviruses that caused non-suppressible viremia (NSV). Here, we asked if additional clones carrying intact proviruses were present in these 3 individuals.

Three donors on ART for 10-20 years who recently developed NSV were sampled over 1-4 years. PBMCs were subjected to endpoint-diluted multiple displacement amplification (MDA). MDA wells were screened for HIV LTR, psi (Ψ), and RRE. Proviruses containing LTR, Ψ, and RRE underwent full-length HIV sequencing and integration sites analysis. A custom pipeline mapped both discrete and non-discrete (e.g., centromeric) integration sites. Gene expression levels in memory CD4+ T cells were determined using the Human Protein Atlas Database (www.proteinatlas.org).

We identified 7 additional clones carrying sequence-intact HIV proviruses (10 total). All 10 integration sites mapped to expressed genes and 4 were in the same orientation as the gene. Five were in KRAB-ZNF genes (50%), compared to the 3.7% of total proviruses in KRAB-ZNF genes in one of the donors ($p<10^{-4}$). Reported gene expression levels were not significantly different between genes with intact vs defective proviruses (median=33.9 vs 61.0 TPM; p=0.3). Predicted-intact proviruses (Ψ+RRE) comprised 9-10% of the total population using the IPDA approach and 2-4% using LTR as the denominator. Proviruses that were confirmed intact by full-length sequencing comprised 0.07-2% of the LTR+ MDA wells.

In 3 donors with NSV on ART, clones carrying sequence-intact proviruses comprised <2% of the total proviral population. All 10 intact proviruses were integrated in genes that are normally expressed in memory CD4+ T cells, including the 5 in KRAB-ZNF genes. Only 1/10 clones declined in size on long-term ART. These results indicate that a stable pool of intact proviruses integrated in expressed genes can persist on long-term ART.

Presenter: Joshua Gluck

Position: Post-Baccalaureate CRTA Fellow

HIGH-LEVEL RESISTANCE TO INTEGRASE INHIBITORS CONFERRED BY MUTATIONS OUTSIDE INTEGRASE

[1]Yuta Hikichi, [1]Sherimay D. Ablan, [1]Erin Clark and [1]Eric O. Freed

Affiliations
[1] Virus-Cell Interaction Section, HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD

Abstract:
Second-generation integrase (IN) strand transfer inhibitors (INSTIs) are highly potent antiretroviral compounds that exhibit a high genetic barrier to resistance. Recent clinical studies concluded that some INSTI-treated individuals experience virological failure in the absence of resistance mutations in IN. The aim of the study is to elucidate INSTI resistance mechanisms and pathways. To examine the pathway(s) by which HIV-1 develops high-level resistance to the INSTI dolutegravir (DTG), we performed long-term passaging of HIV-1 over nearly one year with an escalating concentration of DTG. In a manner independent of viral isolate and coreceptor usage, HIV-1 became resistant to DTG by sequentially acquiring mutations in Env, Gag-nucleocapsid (NC), and, occasionally, IN. By cloning *env* from the DTG-treated viruses selected in the SupT1 T-cell line or PBMC, we obtained heavily mutated Env clones, 7XEnv and WD-3, respectively. Both Env mutants exhibit faster-than-WT replication in spreading infection. 7XEnv exhibits resistance to multiple classes of antiretrovirals, with the fold resistance being ~2-logs higher for INSTIs than for other classes of drugs. WD-3 displays 5-fold resistance to DTG in PBMC. Viral transmission of 7XEnv through cell-cell contact is more efficient than that of WT. In contrast, WD-3 exhibits more efficient cell-free infection than WT. These results suggest that the selected Env mutations confer resistance to INSTIs by increasing infection capacity through cell-cell transmission or cell-free viral infection. Viral infection over a range of multiplicities of infection (MOI) revealed that INSTIs are readily overwhelmed by high MOI, leading to high-level resistance to INSTIs. The NC mutations selected with DTG conferred modest resistance to INSTIs. These findings demonstrate that mutations in multiple regions in the HIV-1 genome collectively contribute to INSTI resistance. The results provide clues to understanding high-level resistance to INSTIs and support the need for genotypic analysis outside of IN in individuals on INSTI-containing regimens.

Presenter: Yuta Hikichi,
Position: Visiting Fellow

HIV-1 uncoating requires reverse transcription of long double-stranded DNA

Ryan C. Burdick[1], Michael Morse[2], Ioulia Rouzina[3], Mark C. Williams[2], Wei-Shau Hu[4] and Vinay K. Pathak[1]

[1]Viral Mutation Section, HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute at Frederick; Frederick, Maryland, USA. [2]Department of Physics, Northeastern University; Boston, MA 02115, USA. [3]Department of Chemistry and Biochemistry, Center for Retroviral Research and Center for RNA Biology, Ohio State University; Columbus, OH 43210, USA. [4]Viral Recombination Section, HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute at Frederick; Frederick, Maryland, USA.

Abstract

HIV-1 cores, which contain the viral genome and replication machinery, must disassemble (uncoat) during viral replication so that the reverse transcribed viral DNA can gain access to the host chromatin and integrate to form a provirus. Our recent live-cell imaging studies have indicated that intact viral cores enter the nucleus and uncoat near (<1.5 μm) the genomic integration site <1.5 hours before integration. However, the viral and host factors that trigger the uncoating of nuclear viral cores remain unidentified. Here, we show that efficient uncoating of nuclear cores requires the synthesis of a double-stranded (ds) DNA genome >3.5 kb and that the efficiency of uncoating is positively correlated with genome size. We studied the replication of a small (3.1 kb) GFP-reporter genome and found that although its reverse transcription was efficient, it was defective for uncoating, integration, and GFP reporter gene expression. HIV-1 core disruption with capsid inhibitors PF74 or lenacapavir induces rapid (<2 hours) viral DNA degradation, indicating that the intact core safeguards viral DNA. DNA of a short vector (3.1 kb) that survives degradation after disruption of cores with capsid inhibitors efficiently integrates within ~2 hours, indicating that the main obstacle to its replication is a failure to uncoat. Atomic force microscopy and viral core content estimation reveal that synthesis of full-length genomic dsDNA, but not small dsDNA genomes, induces significant capsid strain to promote uncoating. These findings indicate that HIV-1 cores protect viral DNA from degradation and that reverse transcription of long dsDNA is required to trigger efficient HIV-1 uncoating. Our data support a model in which HIV-1 evolved to ensure that capsid integrity is maintained until just before integration to deliver the DNA to preferred genomic sites for integration and protect the viral DNA from host DNA nucleases.

Presenter: Ryan C. Burdick
Position: Biologist/Graduate Student

Structure-guided Optimization of HIV Integrase Strand Transfer Inhibitors with Improved Efficacy Against a Broad Panel of Viruses Having Resistant Mutant Forms of Integrase

Xue Zhi Zhao,[1] Steven J. Smith,[1,2] Kuei Chien Tang,[1] Arvin Karbasi,[1] Dario Oliveira Passos,[3] Min Li,[4] Mathieu Métifiot,[5] Daniel P. Maskell,[6] Valerie E. Pye,[6] Peter Cherepanov,[6] Yves Pommier,[5] Robert Craigie,[4] Dmitry Lyumkis,[3] Stephen H. Hughes[2] and Terrence R. Burke, Jr.[1]

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[4]*National Institute of Diabetes and Digestive Diseases, National Institutes of Health, Bethesda, MD, 20892, USA*
[5]*Developmental Therapeutics Branch and Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*
[6]*Chromatin Structure & Mobile DNA Laboratory, The Francis Crick Institute, 1 Midland Road, London, NW1 1AT, UK*

ABSTRACT

Integrase (IN) plays a key role in HIV-1 infection by catalyzing the insertion of viral DNA into host chromatin. FDA-approved integrase strand transfer inhibitors (INSTIs) include the first-generation agents raltegravir (RAL) and elvitegravir (EVG) and the second-generation drugs, dolutegravir (DTG), bictegravir (BIC), and cabotegravir (CAB). These INSTIs are widely used as first-line antiviral therapies. Although second-generation INSTIs have shown improved clinical success against integrase mutations compared with first-generation INSTIs, mutations that diminish the efficacy of the second-generation INSTIs have arisen and the mechanisms by which these mutations cause resistance are often poorly understood. We have developed noncytotoxic naphthyridine-based INSTIs that retain low-nanomolar antiviral potencies against HIV-1 variants harboring the major INSTI-resistant mutations. By analyzing crystal structures of these inhibitors bound to prototype foamy virus (PFV) intasomes, we found that the most successful inhibitors exhibit striking mimicry of binding interactions shown by uncleaved viral DNA and target host DNA. Based on these observations, we proposed a concept of “bi-substrate mimicry,” as an extension of the "Substrate Envelope" principle, in which the efficacy of INSTIs against resistant mutants can be enhanced by mimicking aspects of both bound target DNA and viral DNA, and by filling the substrate-binding regions of the catalytic site. We also examined the effects of substituents at different positions on our naphthyridine-based INSTIs. With the 6- substituted compound **XZ426** retaining better potency against a broad panel of known INSTI-resistant mutants than other analogs that we have described. With a 5-hydroxylmethyl group, compounds **XZ440** and **AK01** also show improved profiles against certain IN mutants. We analyzed the crystal structures and cryo-EM structures of PFV intasomes or HIV-1 intasomes bound to these compounds. We observed that several structural features contribute to their favorable interactions with both the mutant forms of IN and the DNA substrate. These interactions may contribute to their improved profiles against the mutants.

Presenter: Xue Zhi Zhao
Position: Senior Associate Scientist

HIV-1 Transcription Start Site Mutations Negatively Impact Selective Genome Packaging.

Jonathan P. Kitzrow[1], Olga A. Nikolaitchik[1], Saiful Islam[1], Akhil Chameettachal[1], Vinay K. Pathak[2], Wei-Shau Hu[1].

[1]Viral Recombination Section, [2]Viral Mutation Section, HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD 21702

HIV-1 uses RNA polymerase II (Pol II) to transcribe its provirus. The unspliced HIV-1 RNA serves as a translation template to generate Gag/Gag-Pol polyproteins and as the viral genome in virions. There are three consecutive guanosines downstream of HIV-1 promoter and Pol II can initiate transcription from all three guanosines, generating RNAs containing either one (1G), two (2G) or three (3G) 5' guanosines. The number of 5' guanosines heavily influences the function of HIV-1 unspliced RNAs because the 1G RNA is selectively packaged into progeny virion over 3G RNA although these two RNAs only differ by 2-nt. Nearly all HIV-1 variants have three consecutive guanosines downstream from the promoter indicating they are highly conserved. To investigate the impact of these nucleotides on HIV-1 RNA functions and on viral replication, we constructed 8 substitution mutants by replacing one or more of the guanosines. We found that all mutants are infectious and analyzed the transcription start sites in infected cells using 5' rapid amplification of cDNA ends (5'RACE) followed by next generation sequencing. Our results showed that Pol II preferentially initiates HIV-1 transcription using A or G residues but not T or C residues. We then examined the two functions of unspliced HIV-1 RNA: translation and genome packaging. Our findings showed that Gag/Gag-Pol expression levels in all of the mutants are similar to that of the wild-type virus. However, RNA packaging efficiencies of several mutants display severe packaging defects compared to that of the wild-type virus. Taken together, these results demonstrated that the three consecutive guanosines serve important roles in generating multiple HIV-1 RNA species with distinct functional roles. The 5' context of the HIV-1 unspliced RNA does not affect translation efficiency but has a drastic impact on its function as the RNA genome packaged into viral particles.

Presenter: Jonathan Kitzrow

Position: Postdoctoral Fellow

VACCINATION WITH REPLICATION-DEAD MURINE GAMMAHERPESVIRUS PROTECTS AGAINST WILD-TYPE INFECTION AND REACTIVATION IN MICE

MITRA D[1], OLDENBURG D[2], FORREST JC[3], KRUG LT

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[2]Gundersen Medical Foundation: Virology Research, La Crosse, WI, USA
[3]Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

Background:
Gammaherpesviruses are oncogenic pathogens that establish lifelong infections. There are no FDA-approved vaccines against Epstein-Barr virus or Kaposi sarcoma-associated herpesvirus. Murine gammaherpesvirus 68 (MHV68) infection of mice is a system for investigation of gammaherpesvirus pathogenesis and vaccine strategies. We generated a replication-dead virus (RDV) that does not express the essential replication and transactivator protein (RTA) encoded by *ORF50*, leading to limited viral gene expression upon *de novo* infection.

Experimental approach and findings:
Prime-boost vaccination of WT C57BL/6 mice with MHV68 RDV-RTA (ORF50.Stop) elicited virus-specific immune responses and led to a near complete block in virus replication and reactivation from latency. Next, we generated an RDV vaccine lacking the non-coding tRNA-miRNA-encoded RNAs (TMERs) 6, 7, and 8 and the unique M1-M4 genes that promote latency and reactivation of MHV68 *in vivo*. Prime-boost intraperitoneal vaccination of mice with ORF50.StopΔM1-M4 stimulated effector T cell responses in lungs and spleens, respective sites of acute replication and latency, that were comparable to ORF50.Stop vaccination. Vaccinated mice exhibited no splenomegaly and no virus reactivation at 28 days post-boost. When challenged intranasally with WT MHV68, vaccinated mice exhibited near-complete abolishment of virus reactivation from the spleens at 17 days post-challenge.

Conclusions:
These results suggest that major determinants of latency and replicaton are not required components of an effective gammaherpesvirus vaccine.

Presenter: Dipanwita Mitra
Positon: Postdoctoral Fellow

CLONAL EXPANSION AND DRIVING FORCES OF HIV-1 PERSISTENCE IN ANATOMIC COMPARTMENTS

A Glassey[1], W Wang[2], L Adams[1], M Zipparo[1], R Gorelick[3], S Hewitt[4], S Rajan[4], K Lurain[4], R Ramaswami[4], C Lau[1], X Wu[2], B Luke[5], SC Patro[2], T Nguyen[1], F Maldarelli[1]

1. HIV Dynamics and Replication Program, NCI, NIH, Frederick, MD 21702
2. Frederick National Laboratory for Cancer Research, NCI, NIH, Frederick, MD 21702
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4. Center for Cancer Research, NCI, NIH, Bethesda, MD 20892
5. Leidos Biomedical Research, Frederick, MD 21702

Abstract

Upon infection, HIV quickly disseminates and establishes persistent infection throughout the body. HIV persistence mechanisms in tissues are complex, with viral and local environment interaction contributing to tissue-specific pathogenesis. To investigate the anatomic distribution of HIV populations, we characterized HIV-infected cells in tissues obtained at autopsy from individuals on suppressive therapy.

HIV-DNA was quantified by single copy and multiplexed LTR/*gag* ddPCR. Single genome sequences of proviral *gag* were assessed by average pairwise distance (APD) and Slatkin-Maddison analyses. Proviral integration sites (IS) were obtained to assess diversity and clonal expansion rate/patterns; alpha (Simpson) and beta diversity (Bray-Curtis) indicies were calculated to quantify intra- and inter-tissue diversity of clones.

Eight donors(median age = 50y) expired from comorbid illnesses(5 neoplasms, 1 cardiac disease, 2 infection) underwent autopsy within 3-48 hours. HIV-DNA was present in all tissues with highest concentrations in lymph node(43-720 copies/1e6 cells), and lowest in brain (1-9 copies). HIV-LTR/*gag* quantification revealed diverse proviral structures with variable proportions of *gag*-deleted proviruses(0.1%-87.4%). Across tissues, proviruses harbored variable levels of hypermutations(6.2-44.4%) but were not compartmentalized(APD=0.2%-0.9%). We obtained 914 IS from 3 donors with median (range) of 26 (3-163) IS/tissue. In 2/3 donors, clonal expansion rates were significantly different but not tissue-specific with 0-91.9% of clonal proviruses per tissue. Median (range) intra- and inter-tissue diversity indexes were 0.95 (0.53-0.1) and 0.93 (0.84-1) suggesting non-diverse but distinct proviral populations across tissues. In one donor, we observed significant difference in intra-tissue diversity between neoplastic and non-neoplastic (p=0.03) and in inter-tissue diversity between lymphoid vs non-lymphoid tissues (p=0.002).

During therapy, HIV-infected cells are widely distributed in tissues but subject to differential pressures allowing the selection of proviruses with variable levels of defects and hypermutations. Clonal expansion significantly contributes to the proviral landscape. Our data suggests the role of local immune responses in shaping the anatomic proviral landscape.

Presenter: Annemarie Glassey
Position: Postbac fellow (CRTA)

Manufacturing TCR-T cell therapy treatments targeting HPV E7 in patients with HIV and HPV-associated cancer

Scott M. Norberg, Center for Immuno-Oncology, CCR, NCI, NIH

Abstract: Since the advent of modern antiretroviral therapy, the life expectancy of people with HIV (PWH) in the United States now approaches that of the general population. Despite the improvement in life expectancy, PWH are at a 6-fold increased risk of developing cervical cancer and 20-fold increased risk of developing anal cancer (both caused by HPV) compared to the general population. Adoptive T cell therapy (ACT) is one of the most promising treatment modalities developed over the past several decades. Despite the success of ACT targeting cancer antigens, PWH have been excluded from pivotal studies establishing the safety and activity of this treatment due to concerns around cell manufacturing, immunosuppression, impact on HIV control and hypothetical decreased efficacy. No prospective clinical trials testing T cell receptor (TCR)-T cell therapy targeting cancer antigens in PWH and cancer have been conducted to date.

We performed a series of preliminary experiments in which T cells from an HIV+ donor on ART were engineered to express the E7 TCR (E7 TCR-T cells). We found HIV replication occurs during T cell expansion in the absence of antiretroviral agents. When zidovudine (AZT) was added to the culture media, HIV replication decreased without affecting cell phenotype, cell growth, transduction efficiency or vector copy number. When E7 TCR T-cells were cocultured with target cells expressing the E7 antigen in the context of HLA-A*02:01, we observed a significant production of pro-inflammatory cytokines, suggesting that these cells were functional in the presence of AZT. These preliminary findings support the feasibility of manufacturing potent TCR-T cell products from patients with HIV in the presence of AZT. This process will be used to support a phase II clinical trial testing E7 TCR-T cell therapy in patients with HIV and HPV-associated cancer.

Presenter: Scott Norberg

Position: Associate Research Physician

The Effects of Novel Cereblon-binding Immunomodulators on Primary Effusion Lymphoma

Emma N. Treco, Prabha Shrestha, and Robert Yarchoan
Affiliation: HIV and AIDS Malignancy Branch, Center for Cancer Research, National Cancer Institute, Bethesda MD

Abstract
Kaposi sarcoma herpesvirus (KSHV) causes several tumors including primary effusion lymphoma (PEL), an aggressive non-Hodgkin's lymphoma with poor survival. KSHV contributes to PEL development by inducing viral-encoded and cellular oncogenes, and by downregulating immune surface markers, thus evading detection of tumors by host immune cells. A cereblon-binding immunomodulator (CBI), pomalidomide (Pom) is effective against PEL *in vitro*. Pom functions by binding to a cellular E3-ubiquitin ligase, cereblon, thereby inducing downregulation of growth-promoting proteins such as IRF4, cMyc, and CK1a and by preventing downregulation of immune surface markers ICAM-1, B7-2, and MHC-1. New generation CBIs, iberdomide (Iber) and golcadomide (Golc), show increased affinity to cereblon, more efficient substrate degradation, and enhanced tumoricidal activity in various lymphoma models. Here, we assessed the effects of these novel CBIs (from Selleck) on PEL as compared to Pom. Golc and Iber led to significantly higher growth inhibition of PEL cell lines compared to Pom. The IC50 of Golc was 10-fold lower than that of Iber and 100-fold lower than that of Pom. The levels of IRF4 were lower in PEL cells treated with Golc or Iber compared to those treated with Pom. These data indicate that Golc is more potent and effective than Iber or Pom at inhibiting growth and does so at doses well under its C_{max}. Furthermore, Golc and Iber were able to induce ICAM-1 and B7-2 on PEL cell's surface at comparable or higher levels than Pom. Similarly, Golc and Iber were as good or better at preventing the downregulation of MHC-1 in PEL cells undergoing lytic reactivation compared to Pom. This suggests these new CBIs could potentially enhance immune recognition of PEL. Together, these results show that the newer CBIs are generally more potent than Pom, and therefore, should be considered for study in the treatment of PEL patients.

Presenter: Emma Treco
Position: Postbaccalaureate Fellow (CRTA)

In Vivo Treatment with Insulin-like Growth Factor 1 Reduces CCR5 Expression on Vaccine-Induced Activated CD4+ T-Cells

Massimiliano Bissa[1], Veronica Galli[1], Luca Schifanella[1], Monica Vaccari[1,2], Mohammad Arif Rahman[1], Giacomo Gorini[1], Nicolò Binello[1], Sarkis Sarkis[1], Anna Gutowska[1], Isabela Silva de Castro[1], Melvin N. Doster[1], Ramona Moles[1], Guido Ferrari[3], Xiaoying Shen[3], Georgia D. Tomaras[3], David C. Montefiori[3], Kombo F. N'guessan[4,5], Dominic Paquin-Proulx[4,5], Pamela A. Kozlowski[6], David J. Venzon[7], Hyoyoung Choo-Wosoba[7], Matthew W. Breed[8], Joshua Kramer[8] and Genoveffa Franchini[1]

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The DNA/ALVAC-SIV/gp120 anti-HIV vaccine significantly decreases the risk of SIV_{mac251} in the macaque model. At the heart of its efficacy in the absence of neutralizing antibodies is a delicate balance of pro- and anti-inflammatory immune responses that effectively decreased the risk of virus acquisition. Vaccine efficacy was linked to antibodies recognizing the V2 in its helical conformation, V2-specific ADCC, DC-10 tolerogenic dendritic cells eliciting the clearance of apoptotic cells via efferocytosis, and CCR5 downregulation on vaccine-induced gut homing $CD4^+$ T cells. RAS activation prior to vaccination was also linked to increased vaccine efficacy and induction of protective immune responses.
We hypothesized that the induction of RAS activation during the immunizations could lead to ameliorated immune responses and increased vaccine efficacy.
To test our hypothesis, we conducted a preclinical study in female macaques by administering the DNA/ALVAC-SIV/gp120 vaccination in presence or not of a potent RAS inducer, the Insulin-like Growth Factor 1 (IGF-1). The IGF-1 was administered during each immunization either as plasmid DNA encoding the macaque IGF-1 protein (DNA-IGF-1) and/or the human recombinant IGF-1 protein (Increlex®, Mecasermin, IPSEN). At the end of vaccination, systemic and mucosal immune responses were investigated by several immunological assays. The vaccine efficacy was evaluated by intravaginally exposing the animals to repeated low-doses of SIV_{mac251}.
We found that IGF-1 changed the hierarchy of V1/V2 epitope recognition and decreased both ADCC specific for helical V2 and efferocytosis. Remarkably, IGF-1 also reduced the expression of CCR5 on vaccine-induced $CD4^+$ gut-homing T-cells, compensating for its negative effect on ADCC and efferocytosis and resulting in equivalent vaccine efficacy (71% with IGF-1 and 69% without). In conclusion our data posit that, the balance between the increase and decrease in immune correlates due to IGF-1 administration results in a similar vaccine efficacy in vaccinated and vaccinated+IGF-1 non-human primates.

Presenter: Massimiliano Bissa
Position: Staff Scientist

Poster Presentations

Room F1/F2

Poster 1

Investigation of the impact of ΔV1 DNA/ALVAC/gp120 anti-HIV vaccine candidate and/or intravaginal ring releasing SAMT-247 microbicide on the vaginal microbiota

Sophia Brown[1], Massimiliano Bissa[1], Mohammad Arif Rahman[1], Emmanuel Woode[1], Daniel H. Appella[2], Manjula Gunawardana[3], John A. Moss[3], Marc M. Baum[3], Genoveffa Franchini[1].

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According to the 2022 UNAIDS report, women account for 53% of people living with HIV and over 1 million new infections were attributed to young women, especially those living in Sub-Saharan Africa. Therefore, developing effective and innovative strategies to prevent the HIV infection will be essential to curb the epidemic. Our previous studies showed the synergism between the V1-deleted DNA/ALVAC-SIV/gp120/Alum vaccine and the vaginally-delivered gel formulation of SAMT-247 microbicide. The coadministration resulted in over 90% reduction in SIV_{mac251} acquisition in female macaques exposed to SIV_{mac251} intravaginally and showed higher efficacy than the individual administration of vaccine or microbicide. Limitations of this regimen include the limited window of effectiveness of the microbicide administered as a gel and possible low compliance of the users.

Building up on these promising results, we have designed a more user-friendly intravaginal ring (IVR) formulation of SAMT-247 with slow-release capabilities. However, since the vaginal microbiome in women can facilitate HIV infection and impact the effectiveness of preventative vaccines, we hypothesized that our vaccine strategy, the vaginal ring and/or the microbicide could affect the vaginal microbiome composition.

To test our hypothesis, we designed a three-arm study using 34 female macaques to receive vaccine plus SAMT-247 IVR, SAMT-247 IVR only, or Empty IVR, and will investigate the vaginal microbiome and its changes due to the treatments.

In all macaques, vaginal swabs have been collected at baseline, post-vaccination and will later be collected before and after ring insertion and viral exposure. Metagenomic sequencing and inflammatory marker assays will be performed on the swabs to investigate the effects of the regimens on the microbiota. These results will be crucial to understand how the treatments and the microbiota influence each other and impact the vaccine efficacy.

Presenter: Sophia Brown
Positio: Graduate Student

Repurposing antiviral vaccines for local immunotherapy against solid tumors

Shiv K Sethi, Lukas Bialkowski, Claire E Bradley, Yuk Y Pang, Cynthia R Thompson, Douglas R Lowy, John T Schiller, Nicolas Cuburu

Affiliation: Laboratory of Cellular Oncology, CCR, NCI, NIH

Local immunotherapy against solid tumors is considered a viable approach to stimulate the tumor microenvironment (TME) and to promote anti-tumor T cell responses. Here, we interrogate whether licensed subunit anti-viral vaccines could be repurposed to leverage preexisting adaptive anti-vaccine immunity upon intratumoral delivery in a murine tumor model (TC-1) expressing the HPV viral oncogenes E6 and E7. Based on their respective ability to induce CD4 and CD8 T cell responses, we selected Shingrix, a VZV vaccine containing the antigen glycoprotein E (gE) and the AS01B adjuvant (TLR4 agonist and saponin QS21), and Gardasil-9, an HPV vaccine containing the L1 virus-like particles adsorbed on alum

Intratumoral injection of Shingrix in prevaccinated mice delayed tumor growth and often led to complete regression. These responses were associated with the induction of CD8 T cell responses against the HPV16 E7 tumor antigen. The injection of selected MHC-II-restricted gE minimal peptide epitopes combined with polyI:C led to durable remission, suggesting a contribution of gE-specific CD4 T cells. In contrast, intratumoral injection of Gardasil-9 did not delay tumor growth, but the injection of MHC-I-restricted L1 minimal peptide epitopes led to complete and durable remissions. This suggests that minimal peptide epitopes can overcome inefficient class I cross-presentation of native VLP in the presence of preexisting antibodies and promote L1-specific CD8 T cells expansion. TME analysis showed that intratumoral injection of Shingrix with or without Gardasil-derived peptide induced IFN-gamma, TNF-alpha and CXCL9 chemokines and reshaped the myeloid infiltrate from macrophage-dominated toward neutrophils. Finally, intratumoral injection of a combination of Shingrix and a tumor-associated peptide neoantigen led to complete tumor clearance and abscopal responses in a dual flank tumor model.

Our results provide evidence that repurposing anti-viral subunit vaccines as off-the-shelf agents can be effective for local cancer therapy. We show that Shingrix is a versatile platform which can be combined with various vaccine-derived peptides or tumor-associated peptide antigens for in-situ vaccination. This approach could offer a greater access to cancer treatment in low-resource settings and a new blueprint for the optimal delivery of cancer vaccines.

Presenter: Nicolas Cuburu
Position: Staff Scientist

Structural Insights into HIV-1 Vif Degradation of Cell Cycle Regulator PPP2R5A

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HIV-1 virion infectivity factor (Vif) recruits host cullin-RING-E3 ubiquitin ligase and core binding factor beta (CBFβ) to degrade the cellular APOBEC3 (A3) antiviral proteins and avoid inhibition of viral replication by A3-mediated cytidine deamination and lethal hypermutation. Besides antagonizing A3G, A3F, and some A3H haplotypes, Vif also induces G2/M cell cycle arrest by utilizing the same complex to target the regulatory subunits PPP2R5(A-E) of cellular protein phosphatase 2A for degradation. Despite detailed information on Vif-A3 protein interactions, it is unclear how Vif can recognize PPP2R5(A-E) proteins which bear no functional or structural resemblance to the APOBEC3 proteins. Here we report the cryo-electron microscopy (cryo-EM) structure of PPP2R5A in complex with HIV-1 Vif/CBFβ/elongin B/elongin C at a 3.8-Å resolution. Biochemical and mutational analyses support this structure and reveal that the Vif interaction surface of PPP2R5A spans the entire Vif molecule, contacting both the Vif α and α/β domains and partially overlaps with interfaces shared by other A3 proteins. These results increase our understanding of the structural basis of Vif-mediated PPP2R5A substrate recognition and degradation. Development of inhibitors that disrupt the Vif-A3 and/or Vif-PPP2R5A binding interfaces could serve as a new therapeutic strategy to combat HIV-1 infection.

Presenter: Krista Delviks-Frankenberry

Position: Associate Scientist

In vivo detection of HIV-1 antisense transcripts in donors before and during ART

Adam A. Capoferri[1], Toluleke O. Famuyiwa[1], Rachel Sklutuis[1], Sachi Pathak[1], Jennifer L. Groebner[1], Rui Li[2], Jason W. Rausch[1], Steven G. Deeks[3], John W. Mellors[4], John M. Coffin[5], Fabio Romerio[2], Mary F. Kearney[1]

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Abstract

Natural antisense transcripts are expressed by viruses, prokaryotes, and eukaryotes and primarily function in regulating sense gene expression. *In vitro* studies have shown HIV-1 expression of antisense transcripts (AST) from a Tat-independent negative sense promoter in the 3'LTR. In cell lines, AST was demonstrated to promote HIV-1 latency through epigenetic histone modification in the 5'LTR by Polycomb Repressor Complex 2 (PRC2). Here, we asked whether HIV-1 AST is expressed in infected peripheral blood mononuclear cells (PBMC) collected from untreated and ART-treated donors. PBMC were obtained from 11 donors who were either ART-naïve, undergoing treatment interruption, or were virally suppressed on ART. AST levels were measured by single-genome sequencing (SGS) (limiting dilution PCR) and/or digital PCR. We detected HIV AST in 10/11 donors (median of 11 [IQR 2-36] copies/100 infected PBMC). SGS revealed that about 5% of the infected PBMC had detectable levels of AST at a given point in time. HIV-1 genetic diversity of AST was consistent with expression from a diverse population of proviruses. When examining untreated donors with varying levels of plasma viremia, we measured similar levels compared to donors on ART with levels of AST expression by digital PCR. Our findings show that HIV-1 AST is expressed at low levels, similar to other natural antisense transcripts. *In vivo* expression of HIV-1 AST irrespective of treatment status warrants further investigation into its potential role as a long non-coding RNA capable of regulating HIV-1 sense gene expression and inducing HIV latency. Understanding the role of HIV AST *in vivo* may inform future strategies for controlling HIV replication without ART.

Presenter: Toluleke O. Famuyiwa
Position: iCURE Postdoctoral fellow

Neutral Sphingomyelinase 2 Required for HIV-1 Maturation: Selecting for Resistance

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HIV-1 assembly occurs at specific membrane microdomains of the plasma membrane (PM) called lipid rafts, regions typically high in cholesterol, sphingomyelin, and ceramide. Sphingomyelinases (SMases) are key enzymes that produce ceramide through the hydrolysis of sphingomyelin. Neutral sphingomyelinase 2 (nSMase2) is the primary sphingomyelinase in mammalian cells that generates ceramide at the PM. In our recent study, we have shown that inhibition of nSMase2 via either a highly potent and selective inhibitor, phenyl(R)-(1-(3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazo[1,2-b] pyridazin-8-yl)pyrrolidin-3-yl)carbamate (PDDC), or siRNA knockdown disrupts HIV-1 Gag and GagPol polyprotein processing, thereby inhibiting virion maturation and infectivity (Waheed et al., PNAS 2023). However, the precise mechanism by hich nSMase2 inhibition blocks viral protein processing and maturation remains unknown. To this end, we propagated HIV-1 in immortalized T cell lines in suboptimal concentrations of PDDC. We were able to select for and identify mutations in the matrix (MA) and capsid (CA) domains of Gag that confer partial resistance to the nSMase2 inhibitor. We also identified mutations in the protease enzyme that confer PDDC resistance. Current work is focused on elucidating the mechanism by which Gag and protease mutations confer resistance to inhibition of nSMase2. These studies will help to further clarify the role of nSMase2 in HIV-1 maturation.

Presenter: Lindsay Farr
Position: Postbaccalaureate Fellow

Eph receptor tyrosine kinases are entry receptors for the murine gammaherpesvirus 68

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Background. Interactions between viral glycoproteins and cellular receptors determine virus tropism and represent promising targets for vaccination approaches. For the human oncogenic gammaherpesviruses Kaposi sarcoma herpesvirus (KSHV) and Epstein-Barr virus (EBV) entry into target cells is mediated by interaction of the viral gH/gL glycoprotein complex with Eph receptor tyrosine kinases. The Eph-gH/gL interaction is well-characterized in cell culture systems, but its contribution to infection *in vivo* is not well-defined, partly due to the lack of established, naturally susceptible small animal models. Murine gammaherpesvirus 68 (MHV68), a natural pathogen of rodents, is used as an *in vivo* model system for KSHV and EBV.

Study Design and Results. This study aims to test the feasibility of using MHV68 infection of mice as model for Eph-dependent KSHV and EBV infection. We analyzed the interaction of the MHV68 gH/gL complex with Eph receptors using pulldowns of overexpressed protein. MHV68 gH/gL exhibited binding to A- and B-type Eph receptors. Among those were EphA4, an Eph receptor used by KSHV, and EphB3, a high affinity receptor of the related rhesus macaque rhadinovirus. Pre-incubation of MHV68 inocula with soluble decoy receptors decreased infection of permissive fibroblasts while ectopic EphA4 and EphB3 expression enabled MHV68 infection of normally non-permissive B cells. The gH/gL-Eph interaction is dependent on a structural motif formed by domain I of gH and gL, reminiscent of the receptor binding site on the natural ligands for Eph receptors. A targeted alanine screen of MHV68 gH domain I based on homology to KSHV and EBV interacting residues and structural predictions of the MHV68 gH/gL complex bound to Eph receptors identified several residues that play a role in Eph binding. These results will guide the construction of Eph-binding negative MHV68 mutants, which will be used for an in-depth analysis of the Eph contribution to infection *in vivo.*

Presenter: Anna Grosskopf
Position: Postdoctoral Fellow

Role of NK, CD8+ and monocytes in HTLV-1 acute and chronic infection

Anna Gutowska[1], Sarkis Sarkis[1], Arif Mohammad Rahman[1], Katherine Goldfarbmuren[1], Ramona Moles[1], Massimiliano Bissa[1], Melvin Doster[1], Robyn Washington-Parks[1], Katherine McKinnon[2], Isabela de Castro Silva[1], Luca Schifanella[1], Genoveffa Franchini[1] and Cynthia A. Pise-Masison[1]

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Abstract:

Monocytes are professional antigen presenting cells and are at the front line in the defense against virus infection. We previously demonstrated that transient depletion of monocytes prior to HTLV-1 infection enhanced wild type (WT) virus infection. Although one of five animals became infected with HTLV-1 *orf-1* knockout (p12KO) virus, neither WT nor p12KO viral infections were sustained. To further investigate the role of monocytes in early infection, we treated rhesus macaques with monoclonal antibody M-T807R1 together with Clodrosome to deplete these cell populations prior to exposure to lethally irradiated WT or p12KO virus producing cells.

Triple depletion resulted in a rapid seroconversion and high p24Gag antibody titers in all animals. Moreover, the viral DNA level was also increased compared to depletion with either M-T807R1 or Clodrosome as single agents. Finally, infection caused activation and an inflammatory immune response with high levels of IL-6, IL-10, IFNg and IL-12/23. Cytokine profiles in the plasma of infected animals indicated higher increases in sCD40L, IL-13, IL-18, MIP1a and IL-1b in WT compared to p12KO infected animals. Next, we re-treated infected macaques with M-T807R1/Clodrosome to determine if re-depletion of innate cells would make the infection more acute. Re-treatment of infected animals with M-T807R1/Clodrosome, resulted in undetectable NK and CD8+ cells that repopulated by 1 week and 5 weeks post treatment, respectively. Notably, the p24Gag antibody titers and viral DNA detection increased in at day 0 for the majority of animals. Increases in TNFa and IL-8 producing monocyte subsets, macrophages and neutrophils are induced by HTLV-1 infection (both WT and p12KO) in the blood. However, p12KO infection resulted in higher percentages of pro-inflammatory monocytes and macrophages compared to WT infection. Re-depletion with M-T807R1/Clodrosome reduced the difference between p12KO and WT infected animals.

Together these results suggest that NK, CD8+ and monocytes restrict early HTLV-1 infection but play less of a role in chronic infection. In addition to evading CTL and NK killing, the viral Orf-I protein influences the host immune response to viral infection with regards to cytokine and inflammatory monocyte profiles.

Presenter: Anna Gutowska
Position: Postdoctoral Fellow

RELATIVE ACCUMULATION OF DELETED PROVIRUSES DURING ANTIRETROVIRAL THERAPY

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HIV persistence during antiretroviral therapy (ART) is the main obstacle to viral eradication and a barrier to developing curative strategies. Intact and deleted proviruses persist during ART and undergo clonal amplification. The relative proportion of intact HIV proviruses decreases during ART, while the mechanisms responsible for this shift remain uncertain. Previously, we and others reported factors associated with persistence of *gag* during ART. To investigate mechanisms of persistence of *gag*-deleted proviruses, we analyzed levels of *gag*-deleted proviruses in the context of clinical and immunologic characteristics in cross-sectional samples from persons with HIV (PWH) on long-term ART. Clinical information, including immunophenotyping, and peripheral blood mononuclear cells (PBMCs) were obtained from PWH enrolled in IRB-approved protocols who underwent ART for ≥ 3 years. HIV LTR and *gag* DNA levels were quantified by multiplexed ddPCR and analyzed with clinical information by parametric statistics. Participants (N=73, 11% female, median age 50 years [range 20-70 y], median CD4 = 689 [range 250-1765 cells/µl]) had undergone continuous ART for median 14.4 y (range 3-29 y). Over 90% of participants had LTR:*gag* ratios>2 (average LTR:*gag* DNA ratio 5.1), indicating *gag*-deleted proviruses are common after long term ART. Total levels of proviruses varied c. 1000-fold (median 3.4, range: 1.5-4.4 $\log_{10}$ copies/million CD4 cells); levels of *gag*-deleted proviruses (median 2.7, range: <1-4 log10copies/million CD4 cells) were present across the entire range of proviral abundance, and represented as much as 50% of all proviruses in some PWH. No significant correlations between the proportion of *gag*-deficient proviruses as a fraction of total proviruses and demographic characteristics or levels of CD4 or CD8 subsets were detected ($p>0.17$ for all comparisons). These data indicate that *gag*-deleted proviruses are common at all levels of proviruses present in PWH during ART. Lack of immune correlates suggests *gag*-deleted proviruses do not elicit potent cellular immune responses.

Presenter: Danielle Konlian
Position: Postbaccalaureate Fellow

HIV-1 Capsid-Nucleoporin Interactions That Facilitate Nuclear Import of Viral Core

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We recently observed that intact HIV-1 cores are imported through nuclear pore complexes (NPCs) and uncoat in the nucleus near sites of integration. Interactions between the viral core and several NPC proteins, including NUP358, NUP153, and others are critical for HIV-1 nuclear import. Furthermore, only a single FG motif in host cleavage and polyadenylation specificity factor 6 (CPSF6) and in NUP153 are essential for interacting with HIV-1 core and facilitating its nuclear import. Despite these advances, how an intact HIV-1 core navigates through the NPC into the nucleus remains a poorly understood yet critical step during HIV-1 replication.

To identify host factors that modulate HIV-1 core nuclear import, we performed a high-throughput imaging-based siRNA screen of nuclear envelope associated genes and nuclear epigenetic factors. After siRNA knockdown, HeLa cells were infected with HIV-1 labeled with host restriction factor APOBEC3F fused to mNeonGreen fluorescent protein, which incorporated into the viral cores, and viral core nuclear import events were imaged using high-throughput confocal microscopy. A custom MATLAB image analysis pipeline was utilized to determine the efficiency of nuclear import. Subsequent siRNA knockdown of candidate hits showed that NUP98 depletion resulted in potent inhibition of HIV-1 nuclear import (>60%), indicating that it is a strong facilitator of HIV-1 nuclear import.

Co-pelleting assays showed that NUP98 binds to HIV-1 capsid nanotubes. We sought to determine whether, similar to CPSF6 and NUP153, NUP98 interaction with HIV-1 cores is dependent on only one of the 39 FG motifs in NUP98. To identify the NUP98 FG motifs that interact with HIV-1 cores, we established a TRIM5α fusion assay. First, three fragments of the N-terminal 500 amino acids of NUP98 encoding 17, 7, and 15 FG motifs were used to replace the SPRY motif of TRIM5α; all three fragments possessed FG motifs that bound to viral cores and inhibited virus infectivity. Further extensive mutational analyses identified 8, 3, and 9 FG motifs in the respective fragments that were essential for binding to viral cores. These studies show that, unlike the single FG motif-viral core interactions that facilitate CPSF6 and NUP153 binding, multiple FG motifs of NUP98 are required for capsid binding. These studies reveal new insights into how viral core interactions with NUP98 facilitate HIV-1 nuclear import.

Presenter: Sushila Kumari
Position: Postdoctoral Fellow

Visualizing The Dynamics Of Antiretroviral Treatment Interruption *In Vivo*

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Abstract: (297/300)
HIV persistence in anatomic compartments remain poorly understood. Previous analytic treatment interruption (ATI) studies revealed little metabolic activity in lymphoid tissues by 18F-fluorodeoxyglucose Positron Emission Tomography (FDG-PET) during antiretroviral therapy (ART), but increased activity after ATI. Most ATI studies imaged lymphoid tissue and analyzed HIV populations at rebound viremia after many replication cycles. To investigate early HIV reactivation events in lymphoid tissue, we are imaging and sampling lymphoid tissue prior to and following a short ATI. Twelve individuals undergoing long-term ART will be randomized to ATI or continued ART; FDG-PET will identify areas of metabolic uptake for PET-fusion guided lymphoid sampling and HIV population studies at baseline, 10-dayATI, and after ART resumption when plasma HIV RNA <30 c/ml. Metabolic activity is quantified as standardized uptake value (SUV). Participant 1 was a 42-year-old male undergoing ART for 5 years with CD4 562 cells/µl (nadir 7 cells/µl). Metabolic activity was present in nodes of the neck, thorax, and abdomen before ATI; SUV_{max} = 6.4. No metabolic activity differences were detected between baseline, 10-day ATI and re-suppression scans. Single copy HIV quantification demonstrated levels of plasma HIV RNA increased from baseline to 10-day ATI and remained elevated after ART resumption and HIV RNA re-suppression. Participant 2 was a 65-year-old male undergoing ART for 17 years with CD4 590 cells/µl (nadir 165 cells/µl). FDG-avidity was present in paratracheal and bilateral inguinal nodes; SUV_{max} 3.51. The patient was randomized to continued ART. Follow up FDG-PET did not show variation in activity. Metabolic activity persists in lymph nodes during ART and 10-day ATI does not invariably yield increased activity. While HIV RNA in metabolically active lymph nodes reflects ongoing transcription from the provirus, metabolic activity may reflect viral activity of inflammation. Analysis of HIV populations will inform understanding of HIV persistence and reactivation.

Presenter: Chuen-Yen Lau
Position: Research Clinician

Solo LTR formation promotes elimination of proviruses in persons living with HIV

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Abstract:

A major obstacle for curing persons living with HIV (PLWH) is persistence of HIV-infected cells during combination antiretroviral therapy (ART). Most persistent cells harbor defective proviruses, including solo LTRs, which are the result of cellular homologous recombination during DNA replication and clonal expansion; the recombination event excises the entire coding sequence of HIV, leaving only a solo LTR provirus. Solo HIV LTRs are reported (Anderson et al., 2020; Botha et al. 2023) and may dominate proviral populations, but little is known about their frequency and persistence during ART. We combined a new screen for solo LTR with single genome sequencing (SGS) and integration site analysis (ISA) to identify and characterize solo LTR in PLWH. Moreover, we developed specific ddPCR to characterize and quantify levels of HIV clones, and designed bridging PCR strategies that amplify solo LTR to validate provirus structure. Analysis of PBMC from PLWH (N=48) with LTR/gag ddPCR revealed > 90% had excess LTRs (median: 832 excess LTRs/million CD4 cells; range <1-21651). Large clones of solo LTRs were identified in two PLWH; quantification of one clone integrated in HORMAD2 (Anderson et al., 2020) with bridging ddPCR demonstrated this clone now persists 10% of total proviruses >20 years after first identified during early ART. In a second PLWH, analysis of PBMC and neoplastic tissue revealed a solo LTR integrated near the FAM9C gene on the X chromosome; As FAM9C is not located pseudoautosomal recombination regions (PARs) where homologous recombination on the X chromosome is restricted, these data demonstrate that cellular mechanisms responsible for homologous recombination are functional outside of PARs. In summary, HIV solo LTR provirus form in vivo and may be prevalent among proviral populations in adults. Understanding solo LTR formation provides a new insight into requirements for eliminating functional viral sequences from host genome.

Presenter: Feng Li
Position: Research Fellow

Kaposi sarcoma patient-derived xenografts exhibit both an expansion of endothelial cells that actively transcribe viral genes and an amplified gene signature of KS tumors

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Background:
Kaposi sarcoma (KS) is an HIV-associated malignancy defined by hyperangiogenesis, inflammatory infiltrates, and endothelial cells infected with KSHV. KS is a major cause of morbidity and mortality in people living with HIV. Current treatments include antiretroviral therapy, chemotherapy and/or the immunomodulatory therapy pomalidomide, but tumor response is varied and relapse is frequent. The exploration of novel therapies is hampered by the lack of a patient-derived preclinical animal model. Here, we characterize patient-derived KS xenografts (PDXs) in immunodeficient NOD/SCID/gamma (NSG) mice.
Experimental Approach and Results:
Sixteen cutaneous KS biopsies were subcutaneously implanted into 1-4 NSG or NOG mice transgenic for human IL-6. Variables that were tested included VEGF supplementation, tumor dissociation prior to matrigel embedding, and human IL-6 expression. Immunohistochemistry staining for KSHV LANA in spindled CD34+ endothelial cells revealed that KS-PDXs were maintained in recipient NSG for long periods (103 to 272 days), until the experimental endpoint. KS-PDX tumor sizes did not increase in the first or second passage. Regardless of the tested variables and the clinical history of patient volunteers, there was a consistent mean 2.3-fold increase (range 1.2 to 7.1 fold) in KSHV LANA+ of human, NUMA-1+ endothelial cells in the KS-PDX compared to the input biopsies. The Ki67 proliferation marker overlapped with LANA+ areas, consistent with latency-driven cell expansion. Spatial transcriptome analysis confirmed the dramatic expansion of endothelial cells and revealed a broad distribution of viral transcripts from latent and lytic classes across the KS-PDX samples. In addition, signatures genes of Kaposi sarcoma tumors and infected primary endothelial cells were conserved and exhibited a more extreme profile in the KSHV+ regions of the KS-PDX.
Conclusions:
The expansion of KSHV-infected endothelial cells and the recapitulation of the KS tumor gene signature supports the application of patient-derived cutaneous KS xenografts as a pre-clinical model to test novel therapies.

Presenter: Xiaofan Li

Position: Staff Scientist

Distinct Populations of HIV-Infected Naïve and Memory CD4+ T Cell Clones in Children on Long-Term Antiretroviral Therapy

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Pediatric HIV remains a major public health issue with 1.7 million children living with HIV (CLWH) worldwide. Although HIV primarily infects memory $CD4^+$ T cells, recent studies suggest that naïve $CD4^+$ T cells are a significant contributor to the HIV reservoir during antiretroviral therapy (ART). Here, we characterized HIV persistence in naïve $CD4^+$ T cells from CLWH on long-term ART. The cohort consisted of 8 children aged 5-11 years. All children have initiated ART at a median of 9 months of age (range 1.5-17) with suppressed viremia for a median of 8 years. PBMC were sorted into naïve ($CD45RO^-CD28^+CD27^+CD95^-CCR7^+CD45RA^+$) and memory ($CD45RO^+$ $CD95^+$) $CD4^+$ T cells. Multiple displacement amplification (MDA) was used to amplify single proviruses in a background of genomic DNA from the sorted cell populations. MDA products were screened for intactness by amplification of HIV-1 LTR, Psi (ψ) and the Rev response element (RRE). Of the 200 total proviruses analyzed in the naïve T cells, 8 were predicted intact. Intactness was verified in one donor by full length HIV sequencing. All HIV LTR+ MDA products were also used for HIV integration site analyses. HIV infected naïve CD4+ T cells were detected in all 8 children at a median frequency of 38 infected cells/million (range 6-231), a mean of 12-fold lower than the infected memory $CD4^+$ T cells (median 464 cells/million, range 178-506). HIV integration site analyses identified 8 clones of infected naïve T cells, but none of these harbored the predicted intact proviruses. In the child with the greatest depth of sampling (85 integration sites from naïve cells and 174 from memory cells), no matching integration sites were found across subsets, including from the largest infected clones ($p<10^{-4}$). Our findings suggest that naïve and memory T cells may be distinct reservoirs for HIV infection and, therefore, may require different approaches for targeting and eradication.

Presenter: Victoria Neer

Position: Postbaccalaureate fellow

Crosstalk Between Resistance to the HIV-1 Capsid Inhibitor Lenacapavir and Viral Fitness

Binh Nguyen, Alex Kleinpeter, and Eric Freed.
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Abstract

Lenacapavir (LEN) is the first capsid inhibitor to be FDA-approved for HIV-1 treatment. Despite high potency and slow-release kinetics, a significant drawback of LEN is its low barrier to viral resistance. A mutation in the HIV-1 capsid, M66I, confers > 80,000-fold resistance to LEN and has been observed in cultured cells and in HIV-1-infected individuals treated with LEN. However, in the absence of LEN, M66I causes a substantial defect in viral fitness (<5% infectivity relative to WT). Given the high mutation rate of HIV-1, it is important to predict how M66I circumvents this fitness defect before compensatory mutations are manifested in patients. M66I propagation in T-cells repeatedly led to WT reversion (I66M). We next examined the effects of mutating M66 to other amino acids and determined whether these substitutions recapitulate the behavior of M66I, specifically its resistance to LEN and fitness defect. Of the M66 mutants examined, M66L, M66V, and M66F exhibited similar infectivity defects evident in M66I, but only M66V displayed high-level resistance to LEN. Propagation of these M66 mutants led to several second-site mutations. Of note, H12Y, in combination with A105T and other capsid substitutions, resulted in a >10-fold rescue of M66L infectivity. As a clinically significant variant, this work reveals important insights into how HIV-1 may maintain LEN resistance while bypassing the fitness defect induced by the M66I mutation.

Presenter: Binh Nguyen
Position: Postbaccalaureate Fellow

Intact HIV-1 Proviruses are Disproportionally Found in KRAB-ZNF Genes in People Living with HIV on Long-Term ART

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Because antiretroviral therapy (ART) effectively prevents ongoing HIV-1 replication, the HIV-1 reservoir, which primarily comprises CD4+ T cells harboring intact but latent proviruses, is already established by the time of ART initiation and thereafter shaped by selective pressures such as infected cell fitness and propensity toward antigen-driven clonal expansion. It has been anecdotally reported by several groups that after years on ART, intact proviruses are found integrated into Krueppel-associated box Zinc-finger (KRAB-ZNF) genes at frequencies higher than would be expected by chance. Here, considering only integration into genes, we assess the validity of this observation through statistical comparisons among simulated random integration, measured frequencies in primary T cells infected *ex vivo*, and measured frequencies in PBMCs from donors on long-term ART (>10 years). Integration sites were binned by target gene classifications including zinc finger (broadest definition), C2H2-type zinc-finger (ZNF), and KRAB-ZNF genes, and their counterparts defined by exclusion (e.g., non-KRAB-ZNF genes). The resulting aggregate frequencies were then compared across categories and between data sets. Our analysis revealed that normalized integration frequencies in KRAB-ZNF genes were 2.14-fold higher in cells infected *ex vivo* compared to our random integration model ($p < 10^{-5}$) and 1.84-fold higher in cells from donors on ART compared to cells infected *ex vivo* ($p < 10^{-5}$). More significantly, of the ten T-cell clones harboring confirmed-intact proviruses identified in samples from donors on ART, five (50%) were integrated into KRAB-ZNF genes, compared to the 2.37% (307 out of 12,960) KRAB-ZNF gene integration frequency observed for the same samples when all proviruses are considered (i.e., both intact and defective). This more than 20-fold relative enrichment cannot be explained by preferential integration alone. Our quantitative analyses validate and clarify the original reports that intact proviruses integrated into KRAB-ZNF genes are disproportionately represented in the HIV-1 reservoir.

Presenter: Shadab Parvez
Position: Postdoctoral Fellow

V2-specific ADCC rescues SIV vaccine efficacy decreased by mucosal immunization with nanoparticles

Mohammad Arif Rahman[1], Hanna Scinto[2], Massimiliano Bissa[1], Savannah E. Howe[2], Sarkis Sarkis[1], Xunqing Jiang[3], Christina C. Luo[3], Anna Gutowska[1], Luca Schifanella[1], Ramona Moles[1], Isabela Silva de Castro[1], Manuel Becerra-Flores[4], Shraddha Basu[5,6], Kombo F. N'guessan[5,6], LaTonya D. Williams[7,8], Xiaoying Shen[7], Melvin Doster[1], Tanya Hoang[2], Emmanuel Woode[1], Yongjun Sui[2], Georgia D. Tomaras[7,8], Dominic Paquin-Proulx[5,6], Mangala Rao[5], James D. Talton[9], Xiang-Peng Kong[3], Timothy Cardozo[4], Susan Zolla-Pazner[10], Genoveffa Franchini[1], Jay A. Berzofsky[2]

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Deletion of Env-V1 region of the DNA/ALVAC/gp120/alum vaccine regimen improves efficacy compared to envelope-replete immunogens and efficacy is linked to antibody responses to the envelope V2 region. We hypothesized that mucosal vaccination with V2-peptide CKFNMTGLKRDKTKEYNETWYSTDLVCEQGNSTDNESRCYMNHC scaffolded as pentamers with Typhoid Toxin B subunit(V2-TTB) might further improve efficacy, and used poly(DL-lactic-co-glycolic-acid) (PLGA) nanoparticles (NPs) (<100nm) was administered orally to deliver V2-TTB-NPs to the colon.

Rhesus macaques(n=44) were immunized intramuscularly with the standard ΔV1/DNA/ALVAC/gp120/alum vaccine regimen at weeks 0, 4, 8 and 12. At weeks 0, 4, and 16, one group (n=12), also received oral immunizations with V2-TTB-NPs, while other groups(n=9) received TTB-NPs or empty-NPs(n=9). All animals were given 11 weekly intrarectal challenges with SIV_{mac251}.

The addition of V2-TTB-NP to the vaccine did not significantly reduce infection risk compared to the standard vaccine, although among infected animals the peak viral load (VL) and area-under-curve VL(AUC) were both significantly reduced. Surprisingly, no vaccine efficacy was observed in animals vaccinated with the standard vaccine plus either TTB-NPs or empty NPs. V2-TTB-NP group exhibited higher ADCC titers, deltaV1-specific IgA rectal plasmablasts and plasmacytes, and deltaV1-specific IgG in rectal secretions after the last boost. Conversely, TTB-NP or empty-NP-treated animals showed increased plasmacytoid dendritic cells (p=0.056 and p<0.0001, respectively) and IFN-γ^+NKp44$^-$NKG2A$^-$ cells(p<0.0001 and p<0.0001,respectively) compared to that of V2-TTB-NP group. V2-specific ADCC(p=0.01 and p=0.01,respectively), mucosal CD14$^+$ cells(p=0.02 and p<0.0001,respectively) and NKp44$^+$ cells(p=0.004 and p<0.0001, respectively) were decreased in the TTB-NP or empty-NP groups versus V2-TTB-NP group and correlating with protection from acquisition in V2-TTB-NP group.

Collectively the data indicate that despite some stronger mucosal V2- or deltaV1-specific immune responses in V2-TTB-NP group, empty-NPs and TTB-NPs shifted innate mucosal immunity toward a 'non-protective' immune environment for ALVAC-based HIV vaccine and underscore the importance of both quality of innate responses and antibodies to V2 in protection against SIV/HIV infection.

Presenter: Mohammad Arif Rahman
Position: Staff Scientist

Poster 17

Identification of ALVAC, Alum and ALFQA immunological signatures by plasma proteome in vaccinated macaques

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HIV/SIV vaccine efficacy depends on the type of immunogens in the priming phase, the vector and the adjuvant used to formulate the gp120 envelope protein. Our work has led to the development of a reproducibly effective prime/boost anti-HIV vaccine strategy using DNA/ALVAC/gp120/Alum with V1-deleted immunogens. We hypothesized that the vaccine efficacy could be maintained or ameliorated by substituting the ALVAC vectored vaccine with DNA and/or by changing the Alum with the ALFQA adjuvant. To test these hypothesis, male macaques were vaccinated with DNA/gp120/Alum (n=12) or DNA/gp120/ALFQA (n=11), while female macaques were vaccinated with DNA/ALVAC/gp120/ALFQA (n=12) and then compared with the standard regimen DNA/ALVAC/gp120/Alum (n=18) in female. Vaccine efficacy was calculated as risk of virus acquisition of immunized animals versus controls following mucosal exposure of all animals to repeated low doses of SIV_{mac251}. Hystorycal data demostrated no significant difference in virus acquisition in either female or male vaccinated animals, thus these comparison among different groups of animals were validated in spite of the sex and route of challenge differences. Canonical immunological responses and proteomic signatures in plasma and PBMCs were compared among all groups across different time points. We observed a significant reduction in the risk of vaginal SIV acquisition in DNA/ALVAC/gp120/ALFQA group ($p<0.0001$; vaccine efficacy 79%) and the DNA/ALVAC/gp120/Alum ($p=0.007$, vaccine efficacy 65%). In contrast we observed no vaccine efficacy in both groups immunized with DNA, challenged intra-rectally, in the absence of the ALVAC vectored vaccine regardless of the adjuvant used. Principal component analysis of the plasma proteome at 24 hours after the last immunization demonstrated significant differences among vaccine regimens. Additionally, when compared to DNA/ALVAC/gp120/Alum, the substitution with ALFQA induced higher protective immune responses, such as antibody response to variable region 2 (V2) of gp120 and efferocytosis, while decreasing detrimental responses associated with an increased risk of virus acquisition, such as mucosal $NKG2A^{-}NKp44^{-}$ double negative NK cells producing IFN-g. Thus, both vaccine platform and adjuvant impacted HIV vaccine efficacy, underlying a central role for empiricism in the development of effective HIV vaccine candidates.

Presenter: Luca Schifanella

Position: Staff Scientist

Identification of host factors that facilitate nuclear import of HIV-1 cores

Rokeya Siddiqui[1,4], Sushila Kumari[1,4], Ryan C. Burdick[1,4], Gianluca Pegoraro[3], Wei-Shau Hu[2] and Vinay K. Pathak[1]

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Abstract

HIV-1 exploits host cell machinery to replicate, and virus-host interactions can facilitate or inhibit HIV-1 replication. We recently observed that intact viral cores enter the nucleus through nuclear pore complexes (NPCs). Virus-host interactions that influence HIV-1 nuclear import are not well understood. To identify host factors that modulate HIV-1 core nuclear import, we performed a high-throughput, imaging-based siRNA screen of 860 genes from nuclear envelope and nuclear epigenetic libraries. First, after knockdown of the host factors (3 siRNAs/gene), HeLa cells were infected with fluorescently labeled HIV-1. Then, 6 hours post-infection, ~1000-2000 viral core nuclear import events were imaged per well using high-throughput confocal microscopy. Finally, a custom MATLAB image analysis pipeline was utilized to determine the efficiency of nuclear import. From the primary screen, we identified 16 genes based on significant inhibition of HIV-1 nuclear import upon knockdown and validated 15 out of 16 genes with different siRNAs. Interestingly, only 6/15 validated genes encode proteins that are associated with the NPC, indicating that proteins not typically associated with the NPC also directly or indirectly facilitate HIV-1 nuclear import. Two NPC-associated factors (NUP98, NUP160) and three non-NPC associated factors (BRPF3, BRDT, DNMT3A) were further characterized to determine how they facilitate HIV-1 nuclear import. We found that NUP160, BRPF3 and BRDT facilitate stable nuclear envelope docking of viral cores, while NUP98 and DNMT3A facilitate viral core translocation into the nucleus. These studies provide new insights into HIV-1 nuclear import and identify new potential targets for development of antiviral therapies.

Presenter: Rokeya Siddiqui
Position: Post-doctoral Fellow

Antagonism of viral glycoproteins by guanylate binding protein 5

Hana Veler, Geraldine Vilmen, Abdul Waheed, Cheng Man Lun, and Eric O. Freed

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Guanylate binding protein (GBP) 5 is an interferon-inducible cellular factor with a broad antiviral activity, reducing the infectivity of progeny virions by interfering with processing and incorporation of viral glycoproteins. GBP5 is believed to inhibit the infectivity of viruses such as HIV-1, highly pathogenic influenza A, and dengue virus by reducing the proteolytic activity of furin. However, the exact mechanism by which GBP5 inhibits processing of viral glycoproteins and whether it only affects furin-dependent glycoproteins remains poorly understood.

HIV-1 Δ*env* luciferase reporter viruses were pseudotyped with either HIV-1 envelope glycoprotein (Env), MLV Env, vesicular stomatitis virus G glycoprotein (VSV G), SARS-CoV spike (S) or SARS-CoV-2 S glycoprotein and the effect of producer-cell GBP5 expression on particle infectivity was determined. GBP5 was found to reduce the infectivity of particles bearing each of these viral glycoproteins in a concentration-dependent manner. Western blot analysis demonstrated that GBP5 causes a dose-dependent shift in the electrophoretic mobility of the viral glycoproteins in cellular lysates. Moreover, GBP5 strongly reduced glycoprotein incorporation into virions while increasing virion-associated levels of the immature glycoprotein precursors. To confirm that GBP5 affects glycoprotein glycosylation, we treated cell-associated lysates with PNGase endoglycosidase. PNGase treatment resulted in the same mobility shift of glycoproteins as observed in the presence of GBP5, indicating that GBP5 indeed affects protein glycosylation.

These data confirm that GBP5 impairs viral infectivity by interfering with glycoprotein function. Moreover, our work provides evidence that GBP5 not only inhibits furin cleavage of viral glycoproteins but also affects protein glycosylation of both furin-dependent (e.g., HIV-1 and MLV Env and SARS-CoV-2 S) and furin-independent (e.g., SARS-CoV S and VSV G) viral glycoproteins. Furthermore, our data on VSV G indicate that GBP5 targets the glycosylation of proteins other than class I fusion proteins. These results provide novel insights into the broad antagonism of viral glycoprotein function by the cellular host innate immune response.

Presenter: Hana Veler
Position: Postdoctoral Research Fellow (Visiting)

Safety and Pharmacokinetics of SAMT-247 Released via Intravaginal Rings in Rhesus Macaques

Emmanuel K.Woode[1], Mohammad Arif Rahman[1], Massimiliano Bissa[1], Sophia Brown[1], Luca Schifanella[1], Manjula Gunawardana[2], John A. Moss[2], Melvin Doster[1], Rashmi Venkatesh[3], Lisa Miller-Jenkins[4], Ettore Appella[5], Marco Robello[6], Daniel H. Appella[6], Marc M. Baum[2], Elizabeth Glaze[3], Genoveffa Franchini[1].

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In 2022, there were 39 million people living with HIV, 1.3 million people were newly infected and of them, 46% were women or girls. Our prior work demonstrated that combining the ΔV1DNA/ALVAC/ΔV1gp120/alum vaccine platform with vaginal administration of the zinc-finger protein inhibitor, SAMT-247, augment vaccine efficacy in female rhesus macaques. A limitation of the approach, however, is that SAMT-247 was given in a gel formulation, which may not be sufficiently user-friendly, and therefore affect compliance. We have designed a vaginal ring that might overcome this limitation. The goal of the current study is to investigate the drug-release rate and safety of the intravaginal ring (IVR) formulation of SAMT-247 in macaques.

Eight female rhesus macaques were divided into two groups of four. IVR was inserted and maintained in the first group for two consecutive weeks; in the 2nd group for four consecutive weeks. Blood, vaginal and rectal swabs, and pinch biopsies were collected prior to and/or during the study. IVRs were removed and analyzed for residual drug. After three days of ring removal, two of the animals from each group were sacrificed to investigate vaginal mucosal and other tissue pathology to understand the safety of the drug-ring combination.

The IVR consistently provided a steady release of the drug over the course of the study. The *in vivo* drug release rates were 0.97 +/- 0.25 mg/day and 0.84 +/- 0.53 mg/day for the two groups of animals, respectively. Moreover, 81.9 +/- 4.8% and 68.5 +/- 20 % of SAMT-247 was retained in the IVRs after study completion, for groups 1 and 2 respectively. IVR-loaded SAMT-247 demonstrated a great stability profile with 88.0 % and 79.0 % respectively of the remaining drug still detected in parent form.

Administering SAMT-247 at the prescribed rates and duration was safe with excellent hematology, clinical chemistry, body weight, and pathology (cervix, heart, kidney, liver, ovary, oviduct, rectum, urinary bladder, uterus, and vagina) profiles when compared to the baseline controls or reference ranges.

Taken together, IVR-formulation of SAMT-247 shows a steady drug release with a safe profile for up to 4 weeks. Bioanalysis of vaginal and rectal samples are ongoing and will provide further insight into *in vivo* drug concentrations and distribution. The SAMT-247 IVR will be used together with the ΔV1DNA/ALVAC/ΔV1gp120/alum vaccine regimen to assess the synergy of this combination in macaques, with the goal of translating these findings to humans following a GLP-compliant safety study.

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