Current Status and Future Enhancements to Animal Models for AIDS Research

Workshop Report

September 23 – 24, 2019

Hilton Hotel
Rockville, Maryland

NIH National Institutes of Health

Executive Summary

The National Institutes of Health (NIH) Office of AIDS Research (OAR) and the Office of Research Infrastructure Programs (ORIP) convened experts in animal models for HIV/AIDS Research for a 2-day workshop on the "Current Status and Future Enhancements to Animal Models for AIDS Research." The workshop was held September 23–24, 2019, in Rockville, MD, and participants—NIH Institute and Center representatives and investigators seeking to utilize these models for their research—provided the background and up-to-date perspectives to better understand the status of existing and emerging animal models. Participants also identified the gaps in knowledge and resources that limit animal model use in HIV/AIDS research. The agenda included presentations by experts in the field (summarizing key perspectives), panel discussions, and subject matter–focused discussion groups, which culminated in generation of preliminary recommendations for the NIH and the research community on the current, emerging and potential enhancements to animal models for HIV/AIDS research. The workshop had the following objectives:

- Identify the most important enhancements to existing animal models to support HIV/AIDS research.
- Identify the best animal models for specific HIV/AIDS research goals (e.g., stage of infection, particular age groups).
- Identify new and emerging animal models that merit further development and support.
- Determine how best to apply new technologies to improve and support animal models of HIV/AIDS infection.
- Promote sharing of models and samples.

The principal outcomes of the workshop were envisioned to be twofold: (1) a summary report, including recommendations for improvement/refinement of relevant models, to be made available on the OAR and ORIP websites; and (2) a summary opinion piece to be published in a high-impact scientific journal.

The workshop keynote address on **"Considerations in the Development, Optimization, and Application of Nonhuman Primate (NHP) Models for AIDS Research: A Personal Perspective"** emphasized that there are many different NHP models for HIV and that it is important to select the model that most accurately recapitulates the relevant biology of human HIV infection being studied. After touching on the important principle of minimizing the use of animals in research, Dr. Lifson discussed some of the advantages of using animal models, which include experimental control, extensive sampling options, and interventional latitude. Researchers were encouraged to consider the limitations in conducting research studies using the NHP model, including immunologic and practical (e.g., reagent suitability) differences; the viruses used are not HIV; and mucosal challenges do not fully recapitulate natural transmission of the virus. The challenges highlighted were the availability and cost of animals, variability between viral-challenge stocks used by different researchers, the data hurdle for validating new viral challenge strains, and the need for longer follow-up periods to more accurately model some aspects of pathogenesis. NHP models have been instrumental to improving our understanding of the disease mechanisms of HIV and to the development of novel interventions for prevention and treatment. Researchers were encouraged to continue to work to develop and validate new models and to perform thoughtful and well-justified studies in order to continue to advance our understanding of the disease mechanisms and improve preventive and therapeutic interventions for HIV/AIDS.

The remainder of the 2-day workshop consisted of five topical sessions related to the use of animal models for HIV/AIDS research. Each session included five to six short presentations, followed by discussion. Experts in the field reported on new viruses and models, prophylactic vaccines, non-vaccine prophylaxis, HIV cure research, and tools and technologies. The following highlights emerged from the topical sessions.

New Viruses and Models

- There remains a need for age-specific NHP models for SIV in order to study pathogenesis across the lifespan. **The simian immunodeficiency virus (SIV) mac251 model in infant rhesus macaques** is representative of HIV infection in human infants that are not on ART and can be used for pathogenesis, treatment, and cure studies as well as testing the feasibility of early childhood HIV vaccines. This model has also provided insights into virus dissemination, early host immune responses; and the safe use of antiretroviral therapy (ART) in human infants.
- **Knock-in mouse models** expressing either specific or diverse broadly neutralizing antibody (bNAb) precursors are being used to assess the ability of HIV-vaccine candidates/regimens to drive the bNAb responses.
- Early humanized mouse models played a central role in the development of effective ARTs. The next-generation humanized mouse models, such as the **bone marrow–liver–thymus (BLT) mouse model**, allow for better peripheral reconstitution of the human immune response and establishment of high viral loads, which makes it a robust model for assessing cure strategies. Furthermore, researchers are utilizing genetically barcoded HIV strains in the BLT mouse model to facilitate research in HIV latency and persistence.
- Natural hosts for SIV can provide insights into viral-host interactions that are important for disease progression. Studies in **African green monkeys (AGMs)** suggest that the coevolution of with simian SIV has resulted in the downregulation of CD4 gene expression in CD4 T cells. Preliminary studies suggest that DNA methylation may regulate CD4 expression. It was also noted that the ten-eleven translocation (TET) 3 enzyme, which plays a role in the DNA methylation process, is concomitantly down regulated with CD4, suggesting a role for TET 3 in controlling CD4 expression and the potential to develop novel the therapeutics targeting this protein.
- Researchers have developed **simian-human immunodeficiency viruses (SHIVs)** that encode minimally adapted HIV-1 envelope (HIV-1 Env), for testing approaches that target HIV-1 Env. Three novel SHIVs—SHIV.D.191859, SHIV.C.CH848, and adapted SHIV.C.CH505—have been tested in NHPs and show promise for pathogenesis, latency, and cure studies.

Prophylactic Vaccines

- Efforts are ongoing to improve a vaccine in the **SIVmac251 model**. Data show that innate monocyte memory is the most consistent correlate of protection for ALVAC based vaccine.
- A **hybrid cytomegalovirus (CMV) vaccine** engineered to express SIV proteins has been shown to be more than 50 percent efficacious in starkly limiting viremia and preventing disease against highly pathogenic SIV infection. This outcome was only observed with CMV vectors that elicited MHC-E-restricted, SIV-specific CD8+ T cells. Further studies are needed to determine if the unique immunologic characteristics induced by this vaccine can be recapitulated by a human CMV vector.

- Nonheritable factors, such as age, route of birth, rearing practices, CMV status, and obesity have been shown to affect SIV pathogenesis. Researchers are using the NHP model to begin to tease apart the effect of these factors on the immune function. For example, preliminary data suggests that CMV affects the gene expression profile in several cell types including regulatory T cells and dendritic cells.
- A **vaccine** capable of targeting **SIV antigen expression to differentiating epithelial cells** without disseminating into the blood has been developed. Studies in NHPs indicate that immune response induced by this vaccine results in delayed viral transmission, as well as durable control to undetectable viremia over time.
- A dual infection of pegivirus and HIV in humans appears to minimize the effects of HIV infection. A **pegivirus-SIV cynomolgus macaque model** is in development to better understand this protective effect and assess the potential to use pegivirus as a biotherapy for HIV.

Non-Vaccine Prophylaxis

- Reagents are being developed to assess mAbs and bnAbs-based interventions in NHP models. These include first- and second-generation **SIV macaque bNAbs** as well as vectors for antibody delivery. An adeno-associated virus (AAV)-vectored approach was reported to result in stable, long-term Ab expression that protected against low-dose SIV challenge.
- Numerous studies have used NHP SHIV models for testing bNAbs for HIV **pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP)** for HIV. Evidence shows that **passive transfer of HIV bNAbs** prior to exposure can reduce viremia and block infection. Furthermore, antibodies treatments as late as 30 hours after exposure can fully clear infection in newborn and infant macaques. Data suggests that antibodies are most effective when present before the virus is widely distributed.
- The National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center (VRC) and Sanofi researchers co-developed a **trispecific bNAb** targeting three different HIV-1 epitopes that provided enhanced cross-protection and decreased viral escape *in vivo*. In addition, trifunctional T-cell engager antibodies that can target programmed cell death protein 1 (PD-1) or T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domain (TIGIT) in addition to CD3 were well tolerated in chronically SHIV-infected NHPs following a single infusion, leading to redistribution and activation of immune cells in blood and lymph nodes and improved cytolytic activity of CD8 T cells against virally infected cells.
- **Live biotherapeutic products** are being designed to treat, mitigate or prevent diseases in humans. A recombinant live *Lactobacillus jensenii* expressing modified cyanovirin-N (HIV-1 entry inhibitor) was shown to reduce acquisition of SHIV in a female rhesus macaque model.
- The annual global burden of **sexually transmitted infections (STIs)** remains high. **The pigtailed macaque** has similar vaginal and rectal ecosystems to those of humans and are being used to **study multiple prevention technologies for STIs**.

Cure Research in Animal Models

- Clinical cases have demonstrated that bone marrow transplantation with stem cells lacking the C-C chemokine receptor type 5 (CCR5) in HIV positive patients with cancer can lead to a functional cure. Building on these results, researchers have begun modeling cell and gene therapy for an

HIV cure in rhesus macaques. Studies have focused on two approaches to **genetically modify hematopoietic stem cells (HSCs)** to render them resistant to HIV. First is the use of lentiviral vectors to transduce HSCs encoding the C46 peptide form the C-terminal of gp41 that has been shown to inhibit HIV fusion. The second is the disruption of CCR5 using zinc finger (ZFN) editing. Another approach being refined for cure research in NHPs is the generation of HSCs derived **chimeric antigen receptor (CAR) T cells** capable of targeting HIV/SHIV infected cells.

- Preliminary preclinical studies in a fully major histocompatibility complex (MHC)-matched Mauritian cynomolgus macaque allogeneic transplantation model demonstrate that the donor-versus-host allo response depletes the viral reservoir in SIV-infected animals.
- An investigational new drug and **CCR5 antagonist—Leronlimab**—is being evaluated in Phase 2b/3 clinical trials for treating HIV and could be added to an existing therapy in efforts to develop a cure for HIV.
- **Shock-and-kill strategies** to induce **HIV expression from latently infected cells and then trigger cell death** are being assessed in an NHP model. A potential shock-and-kill target is the second mitochondrial activator of caspases (SMAC), which has been shown to promote apoptosis in tumor cells. Recent studies using the **SMAC mimeticAZD5582 suggest that this is a promising strategy to reverse HIV latency**.
- Investigators have developed a **synthetic swarm of SHIV virus with a genetic barcode**, that **can be used to discriminate viral lineages**. Barcoded viral stocks are designed to mimic a diverse population and can be used to model persistent virus over time in rhesus and pigtail macaques. These studies will allow for a more precise assessment of reservoir dynamics. Four barcoded SHIV stocks are available to researchers upon request.
- Vector-mediated antibody expression systems are being developed as an alternative to the traditional vaccine concept. The **BLT humanized mouse model** is being used to assess the efficacy of this approach as well as the potential to generate escape mutants.

Tools and Technologies

Workshop participants described and examined the state of available technologies to support HIV research that could be further optimized for NHP studies. Tools and resources include the following:

- NHP genomics (e.g., B-cell response tracking, comparative genomics, and bioinformatic tools to track antigen [Ag]-specific B-cell response to vaccines).
- Marking bone marrow cells *in vivo* (e.g., clonal tracking of HSCs and/or genetic barcoding).
- Spatial imaging of HIV and SHIV (positron emission tomography [PET] computed tomography [CT]).
- Fluorescently marking virus and bNAbs.
- RNAscope, including development of a computational application to quantify results.
- Repeated biopsy.
- The International ImMunoGeneTics Information System®.
- National Center for Biotechnology Information.

Preliminary Recommendations

The workshop's final activity was an in-depth discussion of current and future needs, touching on knowledge gaps and lack of resources that limit animal models used in HIV research. Participants divided into four breakout groups and discussed key questions, each focusing on one area of future necessities for advancing HIV/AIDS research. Upon reconvening, the group proposed several recommendations.

Most Critical Improvements to Existing Models and Addressing Sharing of Models and Samples

- Develop a central registry for recycled NHP animals (i.e., animals used in prior studies) to be made available to other investigators for method development and/or behavioral studies; consider leveraging the National Primate Research Centers' (NPRCs) secure system in development.
- Consider supplementing base support for animal facilities to reduce the percentage of funding needed for animal acquisition.
- Support research on new SHIVs that replicate more consistently for use in pathogenesis and cure studies.
- Develop a system to ensure that investigators provide NIH the required data on challenge viruses being used to generate stocks prior to sharing the viruses with the broader research community.
- Consider avenues to improve data sharing, such as ensuring study methods and data, including viral stock sequences and pathological data, are transparent to facilitate reproducibility.

Best Models for Each Stage of the Infection Cycle and Different Age Groups

- Provide resources to increase the size of breeding colonies and increase accessibility of NHPs at different age groups for research studies.
- Support research to characterize normal physiological function in understudied age groups; for example, support characterization of the immune systems of infant macaques.
- Continue to develop NHP models for the newborn age group.
- Consider ways to increase studies (e.g., of STIs and drug abuse) in juvenile NHPs.
- Invest in iterative studies between NHP and mouse models; support research to enable exploration of the same mechanisms in both mice and NHPs.
- Develop resources such as humanized mice and colonies of ART-suppressed macaques.
- Develop standard operating procedures for sample collection and preservation.

New or Underutilized Models That Merit Development

- Further expand use of the pigtail macaque model for disease progression, microbicide testing, and reproductive biology.
- Explore using marmosets to examine neuromechanisms associated with HIV infection, including neuroinflammation.

Leveraging Emerging Technologies to Facilitate HIV Research in Animal Models

- Develop a web portal to make available to external investigators samples from ongoing research projects or studies, such as UM1 Cooperative Agreements, in real time.
- Promote inclusion of early-stage investigators in group projects, such as UM1 studies.
- Sponsor training workshops that include travel to laboratories for instruction from experts in protocols and technologies, such as RNAscope, immunofluorescence antibody labeling, and laparoscopic procedures for obtaining biopsies from multiple tissues.
- Consider increasing budgets for R21 Exploratory/Developmental Research grants to align with actual costs of conducting pilot studies in NHPs.
- Consider sequencing for all animals in the following areas: genome, microbiome, rectal, vaginal, B-cell receptors, and T-cell receptors.

DAY 1—MONDAY, SEPTEMBER 23

Welcome and Charge to the Group
Stephanie Murphy, V.M.D., Ph.D., Director, Division of Comparative Medicine, ORIP, DPCPSI, NIH
Nancy Haigwood, Ph.D., Oregon National Primate Research Center (ONPRC)

Dr. Stephanie Murphy welcomed the participants and thanked OAR and ORIP for co-sponsoring the workshop. Dr. Nancy Haigwood commented that work with NHP models has a rich past with an opportune future; scientists have learned much from these models in the past 30 years, and this workshop was convened to discuss potential enhancements to the existing models and determine what additional HIV-specific research goals could be addressed with NHP models. She added that participants should discuss whether any new or emerging models merit further development and support and whether any new technologies could improve use of the model. This meeting also aimed to promote the sharing of models and samples.

Considerations in the Development, Optimization, and Application of NHP Models for AIDS Research: A Personal Perspective
Jeffrey Lifson, M.D., AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Frederick, MD

In the keynote presentation, Dr. Jeffrey Lifson outlined the use of NHP models in the context of HIV/AIDS research.

Decision-Making Process for Using the NHP Model

Dr. Lifson first noted the "three R" principles regarding the use of animals in research ("replacement, reduction, and refinement") but affirmed that NHP models represent the best available experimental systems in which to address certain key, complex, biologically and clinically significant questions in HIV/AIDS research. He reminded participants of the importance of choosing an NHP model that authentically recapitulates the relevant biology of human HIV infection that they propose to study.

Overview of NHP Models

NHP models allow control of external factors and data collection, but fundamental differences between humans and NHPs necessitate practical adaptations and analytical considerations. Dr. Lifson reminded attendees of the purposes of NHP models and discussed the balance between the proliferation and consolidation of animal models, then he provided highlights from his 35 years of work in HIV/AIDS research. Asian macaques, which are not the natural host for SIV but are susceptible to infection, are the most commonly used NHP models for AIDS-related studies and are mainstays for studies of pathogenesis, transmission, prevention, treatment, and—more recently—a "cure." Asian macaque species differ in their availability, natural history, and previously collected data. Furthermore, some species display characteristics that render them well suited to address a particular question. Dr. Lifson also commented briefly on the use of African NHP species, which have endemic SIV. Exposure of these populations to SIV for extensive periods of time has led to co-evolution of virus and host and development of various mechanisms limiting pathogenesis. Dr. Lifson encouraged researchers to select thoughtfully the appropriate species, age, and sex to address the question studied, although animal availability and cost can be significant challenges.

Virus-Challenge Stocks and Experimental Designs

Dr. Lifson discussed the importance of choosing a suitable viral strain with which to challenge NHP models; considerations include virus identity/lineage; isolate/swarm vs. clone; method of preparation; synthetic swarms/barcoded viruses; and the route, dose, and number of challenges. Dr. Lifson spoke also on ensuring that the virus is characterized appropriately, noting that stocks prepared by different laboratories often vary. He proposed a potential minimum required characterization of viral stocks used in NHP studies intended for publication. Parameters proposed for required reporting included—

- Original source/provenance of virus
- How the virus was prepared (starting material; seed virus stock source vs. plasmid; infection/transfection; producer cells)
- Titer (*in vitro*, specify target cell line/assay TZM-bl, CEMX174, Primary CD4/PBMC +/- dextran/polybrene; *in vivo* route, number of animals)
- RNA content with method specified
- p27 content with method specified
- Sequence analysis with method specified

Dr. Lifson spoke briefly on new "designer" viruses and discussed the balance between novel and older, well-established viral stocks. As with model choice, Dr. Lifson emphasized the importance of carefully choosing the best virus for the relevant question and documenting the characteristics of viruses used in each study. He acknowledged the validation data hurdle as a challenge to embracing new and "better" viruses.

Dr. Lifson further underscored the importance of NHP models in increasing researchers' understanding of HIV pathogenesis, noting potential applications to clinical findings that indicate viral replication and intestinal damage are associated with immune activation and pathogenesis. Dr. Lifson conveyed that researchers must ensure that any NHP pathogenesis model recapitulates what is known about *in vivo* pathogenesis in the human setting, recommending iterative, correlative assessments with clinical

colleagues to confirm recapitulation. He recognized challenges regarding the follow-up duration for clinically relevant modeling, as well as incomplete incidence of some aspects of pathogenesis.

Regarding prevention, Dr. Lifson highlighted recent literature demonstrating the protective functions of injected antibodies and antibody-like inhibitors and recommended using NHP models to assess safety, demonstrate proof of concept, and overcome hurdles to clinical evaluation. Dr. Lifson turned next to treatment, recounting several scenarios in which studies in NHPs addressed critical challenges. He stated the importance of using NHP models to assess treatment strategies and noted that his recommendations and the challenges for treatment mirror those for prevention research. Finally, Dr. Lifson discussed targeting the persistent viral reservoir and described his team's efforts to use an NHP model to address this issue. He recommended researchers validate NHP models for clinical relevance when using SHIVs for reservoir or "cure" studies, prevention, treatment, and cure studies. Dr. Lifson noted common challenges for prevention, treatment, and cure studies, which include cost, time, and the debate as to whether NHP studies should be used as a "gatekeeper" (i.e., NHP studies would be required prior to clinical trials). Cost and time are particularly relevant to cure studies because of the need for extended combined antiretroviral therapy (cART) to achieve clinically relevant levels of viral suppression.

Futuristic View for NHPs in HIV/AIDS Research

Dr. Lifson reiterated that NHP models have made—and continue to make—critical contributions to the understanding of disease mechanisms in HIV infection and the development of novel interventions for the prevention and treatment of infection. He suggested that if researchers in the field continue to perform studies thoughtfully—including developing and validating new models—they will develop a better understanding of disease mechanisms and improve preventive and therapeutic interventions for HIV/AIDS.

SESSION 1: NEW VIRUSES AND MODELS

Session Chair: Ann Chahroudi, M.D., Ph.D., Emory University School of Medicine

The Need for Age-Specific NHP Models to Address Clinically Relevant Questions of HIV Infection and Prevention

Kristina De Paris, Ph.D., The University of North Carolina School of Medicine

Dr. Kristina De Paris described HIV pathogenesis across age groups and emphasized that children with HIV experience major damage to their developing central nervous systems (CNS), which can lead to developmental delays. Adolescents living with HIV experience higher substance use rates, which also contributes to CNS damage; in adults living with HIV, the most frequent complications are co-morbidities. The differences in pathogenesis across age groups highlight the need to include infants in NHP studies. The SIVmac251 infant infection model in rhesus macaques can be applied to pathogenesis, treatment, vaccine, and cure studies; has provided insights into virus dissemination and early host immune responses; and has supported safe use of ART in human infants. This model is especially important for studying CNS complications associated with HIV. Whereas the cerebrospinal fluid must be used as a surrogate for human CNS function, NHP models enable researchers to directly view the brain, as well as other tissues that are difficult to sample *in vivo*, particularly in humans. NHP models allow for detailed tissue analysis, identification of HIV-induced insults to the developing brain, definition of the impact of time of ART initiation on neurodevelopment, and determination of whether the brain serves as a viral reservoir. However, NHP models are less useful for characterization of motor skills, memory, verbal function, and social behavior. Studies in infant macaques indicate that SIV-infected animals have reduced neuronal growth in the hippocampus and dorsolateral prefrontal cortex compare to uninfected

animals. Dr. De Paris is also beginning to assess viral reservoirs within the CNS and preliminary data suggest that glia cells can be infected. It will be important to determine viral reservoirs for infants within the CNS as studies examining viral reservoirs in the periphery indicate that are differences between adults and infants. Adult macaque viral reservoirs are found in central memory T cells, but in infant macaques, the majority of viral DNA is found in naïve T cells. The macaque model will enable researchers to determine whether macrophages can serve as viral reservoirs.

Dr. De Paris discussed vaccine strategies to produce anti-HIV-1 Env antibodies. She presented data from oral infections with three SHIVs. All three elicited high acute viremia, but chronic viremia was variable. Long-term pathogenesis studies are needed to determine how these SHIVs compare to human pediatric HIV infections. Further vaccine efficacy studies must account for functional immune differences between infant and adult macaques and optimization of a pediatric vaccine is likely to require an age-appropriate adjuvant. Dr. De Paris emphasized that early vaccination to protect adolescent women against HIV is needed to stop the generational cycle of infection. The NHP model can be used to test whether infant vaccination is protective against sexual transmission of HIV in adolescence. For these studies, scientists need larger group sizes with adequate sex representation and study duration, as well as applying systems biology tools.

Optimizing Mouse Models for Testing HIV Vaccine Candidates
Ming Tian, Ph.D., Harvard University

Dr. Ming Tian discussed optimizing knock-in mouse models for testing HIV-1 vaccine candidates. He characterized two types of mouse models. The first model type expresses the pre-arranged variable region exons of the precursors to bNAbs. These mice possess monoclonal B-cell populations that express the target antibody, and researchers test whether the immunogen can activate B cells after immunization. This model is advantageous for its efficient ability to test whether immunogens are effective. Limitations include high precursor frequency, a lack of competing antibodies, and the need to use a fixed complementarity determining region 3 (CDR3), which is variable in humans. The second mouse model is more physiologically based, expressing diverse bNAb precursors. Researchers insert gene segments for *de novo* rearrangement. This model uses diverse CDR3 and enables multiple antibodies to be compared by the level of response to immunogens, which allows determining whether immunogens are selective for the bNAb epitope. This method still contains limitations because of differences between mouse and human precursor frequency, CDR3 distribution, sets of competing antibodies, and MHC and T-cell response. Dr. Tian concluded that a promising immunogen found using this model would be a candidate for testing in NHPs or clinical trial.

Humanized Mouse Models to Study HIV Pathogenesis
Jerome Zack, Ph.D., University of California, Los Angeles

Dr. Jerome Zack explained that humanized mice are powerful tools for basic and translational research, with many applications. The humanized BLT model is the most robust model currently available to study cure strategies because it generates desirable peripheral reconstitution and high viral loads, and the cells are restricted to human leukocyte antigen molecules. Multilineage hematopoiesis in BLT mice enables detection of human leukocytes, including dendritic cells, natural killer (NK) cells, T cells, B cells, and antigen presenting cells. This robust reconstitution of the human immune system enables applications for gene therapy and drug approaches. It requires fetal tissue; however, political barriers may therefore pose difficulties for this model's continuation. Dr. Zack and his colleagues are improving the model by generating genetically barcoded HIV strains, which facilitates research in HIV latency and persistence.

The relatively short experimental period in humanized mouse models allows researchers to compare, rapidly and relatively inexpensively, strategies for knocking out viral reservoirs.

Epigenetic Modulation of the Cluster of Differentiation (CD) 4 Gene Leads to Downregulation of CD4 in African Green Monkeys
Jason Brenchley, Ph.D., NIAID, NIH

Dr. Jason Brenchley discussed differences between host species in studying disease progression. About 50 species of NHPs in Africa are naturally infected with species-specific versions of SIV; these animals served as the sources for the viruses that cause HIV infection in humans. Dr. Brenchley emphasized that this model is useful because the animals have co-evolved with the viruses to avoid disease progression. Comparing natural-host SIV infection with non-natural pathogenic infection can provide information on which aspects of the infection are important for disease progression. A SIV-infected natural host has a viral load comparable to a human with HIV who has not received ART, but many pathologies are absent. SIV-infected AGMs are the most commonly used model for non-progressive infection. Most researchers study *Chlorocebus sabaeus*, a species of AGM with a small founder population and thus limited genetic diversity. Dr. Brenchley described his work on several species of natural hosts; results indicate that co-evolution with SIV likely involved downregulation of CD4 by epigenetic modulation. This finding could lead to novel therapeutic interventions to downregulate or inhibit the TET3 enzyme in human CD4+ T cells.

Novel SHIVs Encoding Minimally Adapted Transmitted/Founder Envelopes
Katharine Bar, M.D., Penn Center for AIDS Research

Dr. Katherine Bar discussed the uses of transmitted/founder (TF) SHIVs in NHP research. SHIVs remain important for testing envelope-based strategies of prevention, therapy, cure, and pathogenesis. Researchers have developed SHIVs that encode minimally adapted HIV-1 Env, which enable testing of methods to target HIV-1 Env. Dr. Bar and her team have generated at least 20 TF SHIVs that are well characterized both *in vivo* and *in vitro*; these are available for investigators to use. Many of these viruses share fundamental characteristics with HIV-1 Env, including host–pathogen interactions that lead to specific patterns of bNAb or non-bNAb induction in humans. Dr. Bar discussed a particular virus strain, SHIV.C.CH505, that researchers use as a challenge virus for vaccine studies. The human initially infected with this virus developed bNAbs, and a substantial number of infected rhesus macaques exhibit spontaneous control or very low viremia. Therefore, this virus is not currently ideal for long-term studies on pathogenesis or ongoing replication. Researchers aim to minimally adapt this virus to retain some of its antigenic properties but enhance its replication, optimizing it for latency and cure strategies. Dr. Bar offered to provide viral strain generation definitions and additional relevant information to any investigator who would like to use viruses from her laboratory.

Panel Discussion Highlights

- When asked how the utility of mouse models is compared, Dr. Zack explained that new models reconstitute more fully the human immune system and enable longer term experiments. The most appropriate model for any given experiment will depend on the specific research question being investigated.
- A participant asked whether, given the complexities of obtaining human fetal tissues, researchers would consider using fetal NHP tissue for mouse models to explore pathogenesis. Dr. Zack responded that this is possible, but an NHP model would be more robust than the mouse model in

this case. Participants discussed the costs associated with humanized mice versus NHPs and possible sources of tissue for such studies.

SESSION 2: PROPHYLACTIC VACCINES

Session Chair: Genoveffa Franchini, M.D., NCI, NIH

Comparative Vaccine Approaches: Measuring Outcomes in Primate Models

Genoveffa Franchini, M.D., NCI, NIH

Dr. Genoveffa Franchini spoke on the importance of evaluation parameters for determining functionality and efficacy of vaccines in animal models and reported on her group's efforts to develop and improve a vaccine within the SIVmac251 model, noting that they optimized the primers and vectors used. She also reviewed the methodologies employed in investigating the basis of the vaccine's correlates of protection (e.g., traditional immunology, systems serology, toxicity analysis, and microbiome analysis) as well as route of infection (e.g., vaginal versus rectal). She identified innate monocyte memory as the most consistent of the correlative factors, stating that, in her model, the effect induces a nonspecific and sustained response. Additionally, responses differed based on route of entry. She raised the issue of age bias in studies, noting that monocyte function is known to decrease with age. Dr. Franchini observed that the field is beginning to address the need for direct experiments to validate correlations.

Cytomegalovirus Vectors: New Immunobiology Discovered in Rhesus Macaques

Louis Picker, M.D., Oregon Health & Science University

Dr. Louis Picker highlighted the importance of the rhesus macaque as a model organism for basic scientific research—studies of this species complement both studies of lower level organisms and human trials. One challenge of developing vaccines for HIV is that the virus' rapid replication rate renders traditional vaccine designs ineffective and allows the virus to evade the host's immune response. Because CMV elicits a rapid immune response, however, a hybrid CMV vaccine, engineered to express SIV proteins, provokes a sufficient effector differential response. Dr. Picker highlighted the confluence of human-like CMV model and HIV models; because neither is available in a rodent-based model, the discovery of CMV vector efficacy could have been accomplished only in the rhesus macaque model. The vaccine induces unique immunologic characteristics, and vaccine efficacy is present only in vectors that are programmed to MHC-E-restricted T cells that recognize full SIV inserts or "supertype-only" inserts. Dr. Picker explained that these findings provide a pattern of previously unfeasible viral control and posed the critical question of whether human CMV vectors will recapitulate the unique biology and efficacy of rhesus CMV vectors. Because the concept has since been demonstrated in other species, Dr. Picker expressed hope for development of an effective human vaccine based on this principle.

Regulatory T Cells and Antigen-Presenting Cells in the Mucosal Immune System

Dennis Hartigan-O'Connor, Ph.D., University of California, Davis

Dr. Dennis Hartigan-O'Connor discussed possible effects of nonheritable factors on immune function. These factors, including age and birth/rearing factors (e.g., pathogen exposure, obesity, breastfeeding), impart influences known to be important for SIV pathogenesis. Dr. Hartigan-O'Connor commented that although these factors can be a nuisance, they illustrate a unique advantage of working with primates—primate diversity mirrors that of the human population. He discussed work to characterize the effect of CMV infection on innate and adaptive immune system genes, noting that many cell types appear to be affected. Dr. Hartigan-O'Connor currently is working to incorporate these effects within his study designs. His approach comprises awareness in the project-planning and animal-assignment stages,

frequent measurement of immune status by whole-blood staining, and investigation of pre-intervention parameters as predictors of outcome. Vectored immunizations are followed by vaccine antigen-independent immune perturbations with effects that also should be controlled in analysis.

Epithelial Stem Cell-Based AIDS Vaccine: Mucosal Immune Responses and Control of Transmission in Macaques
Marie-Claire Gauduin, Ph.D., Texas Biomedical Research Institute

Dr. Marie-Claire Gauduin discussed the control of viral entry as a strategy to counter the rapid replication rate of SIV. She explained that one key obstacle to an effective AIDS vaccine has been the inability to deliver antigen for a sufficient period of time, leading to weak and transient protection. When the virus enters the host, the primary phase of infection is characterized by viral replication, dissemination from the point of infection, and the immune response. Consequently, viruses accumulate rapidly during the acute phase. Dr. Gauduin has developed a vaccine that restricts viral replication at a mucosal portal of entry and may control HIV transmission. The vaccine uses the epithelial stem cells as a permanent source of viral antigens and their differentiated offspring as antigen-presenting cells without disseminating into the blood, thus inducing strong mucosal antibodies against SIV. Overall, this study demonstrates the efficacy of an epithelial stem cell-based vaccine to serve as an antigen delivery system and generate specific mucosal antibody and cellular immune responses; this effect leads to a significant delay in infection and, consequently, control of viremia to nondetectable levels.

HIV Biotherapy: Lessons From Simian Pegivirus Infection of SIV+ Macaques
David H. O'Connor, Ph.D., University of Wisconsin–Madison

Dr. David O'Connor indicated his talk would focus on "falling forward," by using lessons learned in creating animal models to improve and advance the field. He emphasized the balance between fidelity and pragmatism for the development of animal models, highlighting such issues as sample size, husbandry costs, and timelines. He also described the complexity of selecting appropriate model organisms and pathogens, noting that in some cases, other organisms (e.g., humans, lower animals, bacterial cultures) may be more appropriate for study than macaques.

Dr. O'Connor stated that pegiviruses provide a useful example for thinking about these issues. When humans are infected with a pegivirus and HIV simultaneously, the effects of HIV are minimal; the pegivirus appears to dampen the patient's immune response. Dr. O'Connor described his efforts to create an animal model for both pegivirus and HIV. Although the results of a recent study using cynomolgus macaques did not yield evidence for the protective effect of the pegivirus, the data offered new information on pegiviruses. Dr. O'Connor spoke on the importance of building failure into the experimental design, noting that these failures often yield information that informs and improves the care and use of macaques as animal models. He added that open sharing of data and experimental design will be key to progress within the field.

Panel Discussion Highlights

- Dr. Picker pointed out that Dr. O'Connor's study used only four animals, commenting that one might argue that no conclusions can be drawn from such a small sample size. Dr. O'Connor agreed that such studies are underpowered. He noted that sample size has become an important consideration in recent years because of increases in costs for animal use, as well as ethical issues surrounding the use of large numbers of animals. He emphasized that transparency, although not a solution, will be critical in moving forward.

- When asked which journals would publish studies with negative findings, Dr. O'Connor conceded his privilege as a relatively senior investigator, but he stated that several journals (e.g., *PLOS ONE*) are likely to accept papers that exhibit technical merit in lieu of perceived impact for the field.
- When asked how the costs of animal husbandry could be reduced, Dr. Haigwood replied that most centers have effective breeding programs, and the NIH does not charge investigators for the full cost of raising the animals. Program costs include health care and pedigree analyses. Dr. O'Connor stated that his laboratory has begun using cynomolgus macaques to reduce costs, and he suggested that researchers could save money by purchasing animals raised in other countries. He stressed that the community should evaluate the costs and benefits of continuing to rely on rhesus macaques, as that reliance complicates availability. A participant cited quality issues with animals bred outside the United States and added that the field has accumulated a massive historical data set on the rhesus macaque that informs future studies. It was pointed out that the issue of availability stems from limits to grant funding, as this limits breeding of the animals.
- Dr. Franchini observed that researchers eventually will need to test the HIV immunogen to develop vaccines that elicit antibodies, because the HIV envelope differs structurally from that of SIV.

SESSION 3: NON-VACCINE PROPHYLAXIS

Session Chair: Mario Roederer, Ph.D., NIAID, NIH

SIV and NHP bNAbs to Model Antibody-Based Intervention

Rosie Mason, Ph.D., VRC, NIAID, NIH

Dr. Rosie Mason emphasized the importance of identifying the appropriate model to answer a desired question and the tools necessary to evaluate experiments with that model. She described the tools necessary to investigate an SIV antibody-based intervention using rhesus macaques. She identified antibodies, effector functions, vectors for antibody delivery, and challenge viruses as important components of the SIV NHP toolbox. Dr. Mason described her group's efforts to characterize first- and second-generation SIV macaque bNAbs. She highlighted structural and functional similarities between the SIV model and HIV, and invited participants to request antibodies from her team.

HIV bNAbs as Pre-Exposure Prophylaxis and Post-Exposure Prophylaxis in Primate Models

Nancy Haigwood, Ph.D., ONPRC

Dr. Haigwood provided an overview of studies describing NHP SHIV models for testing bNAbs, noting the breadth of work performed within the field. The virus and the ever-evolving envelope work together to drive antibody responses, and bNAbs have helped identify places in the trimer vulnerable to attack by antibodies. Dr. Haigwood commented on the large difference in the ability of specific antibodies to neutralize the virus *in vivo* or *in vitr*o, which probably is related to the rapidity with which the virus establishes itself in tissues. Envelope variants and antibodies in both HIV infection and NHP models have co-evolved. Pre-exposure with passive antibodies can reduce viremia and block infection. Importantly, she showed that antibodies can also be delivered as late as 30 hours post-exposure and fully clear infection in newborn and infant macaques. Although the antibody has the potential to reach the virus and participate in clearance, whether antibodies are actually able to do this is not yet known. Dr. Haigwood outlined experiments showing that antibodies are likely most effective when present before the virus is

widely distributed. She cautioned participants that the role of SHIV in adult animals may be different but noted that these studies suggest ways to use SHIV models to answer specific questions.

Bispecifics and Engineering of bNAbs *In Vivo*
Amar Pegu, Ph.D., VRC, NIAID, NIH

Dr. Amar Pegu described his group's work using bioengineered bNAbs for prevention of viral infection. Several proof-of-concept studies have shown that bNAbs can prevent SHIV infection, and upcoming studies will address whether antibodies can provide protection against HIV infection. The NIAID VRC is developing next-generation bNAbs for use in HIV-1 prevention that have longer half-lives, increased potency, and greater breadth. Dr. Pegu emphasized that because no single bNAb neutralizes all circulating strains at high potency, targeting multiple sites is required, and cocktails of two or more bNAbs will be necessary. Next-generation antibody technology can be used to develop antibodies with multiple specificities in the same molecule, potentially lowering the costs of manufacturing, delivery, and administration. The VRC helped to engineer a tri-specific bNAb that offers enhanced cross-protection and decreased viral escape *in vivo*. Immunotherapy studies in NHPs showed good viremia control and a rebound strongly correlated with antibody levels, suggesting that suppression is successful when protein levels are maintained. Dr. Pegu also described studies that suggest these antibodies can be used to address the chronic viral infection CD8 exhaustion model.

Use of the Female Rhesus Macaque for Vaginal Microbiome Modification to Test Live Biotherapeutics
Laurel Lagenaur, Ph.D., NCI, NIH

Dr. Laurel Lagenaur's team has been using NHP models to develop live biotherapeutic products designed to treat, mitigate, or prevent disease in humans. Dr. Lagenaur reminded participants that the human vaginal microbiome is unique among primates because it is lower in diversity, with *Lactobacillus* as the dominant genus. A more diverse vaginal microbiome, such as that seen in bacterial vaginosis, increases susceptibility to HIV infection. Macaques have menstrual cycles similar in length to humans and no distinct breeding season in captivity, but macaque vaginal microbiomes are more diverse and thus similar to humans with bacterial vaginosis. Glycogen, a key nutrient for *Lactobacillus*, is much lower in NHPs than humans, thus explaining why NHP vaginal microbiomes are not *Lactobacillus*-dominant. A recombinant, live *L. jensenii* biotherapeutic product, developed for HIV protection, was used to inoculate macaques after treatment with antibiotics. Macaques that received live *L. jensenii* biotherapeutic had a 63 percent reduced acquisition of SHIV. Increases in *Lactobacillus* were accompanied by decreases in *Proteus*, *Bacteroides*, and *Veillonella* species, indicating that specific genera were being suppressed. Dr. Lagenaur stated that all macaque models her team studied could be used to mimic the human vaginal microbiome during bacterial vaginosis.

Pigtailed Macaque Model: Preclinical Safety and Efficacy Assessments of Multiple Prevention Technologies
Dorothy Patton, Ph.D., University of Washington

Dr. Dorothy Patton discussed the high annual global burden of STIs—in particular, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae,* and *Mycoplasma genitalium*—because these STIs and their sequelae disproportionally affect young women. The development of safe, effective barriers, such as multipurpose prevention technologies (MPTs), is urgently needed to prevent both the acquisition and spread of these common STIs. An animal model to evaluate MPTs ideally shares physical and reproductive characteristics with humans and is similarly susceptible to the STIs studied. Such a

model should allow researchers to assess the utility, safety, and efficacy of candidate MPTs and vaccines. The pigtailed macaque model was developed by the Patton laboratory to simulate human *C. trachomatis* infection, including cervicitis, salpingitis, pelvic inflammatory disease, pathogenesis, and disease outcome. Dr. Patton's team expanded the usefulness of this animal model to evaluate the safety and efficacy of disease prevention strategies, including MPTs for vaginal and rectal use and vaccine candidates for the prevention of STIs in humans. Dr. Patton reviewed preclinical studies conducted in the pigtailed macaque model that identified safe formulations of topical MPTs for vaginal and rectal use.

Panel Discussion Highlights

- Participants discussed the potential effects of isotype and glycoformat, suggesting that the humanized mouse model might be better than NHP models to address such questions.
- Participants discussed the difference in response to one or multiple antibodies between SIV and SHIV, emphasizing the importance of discerning whether HIV is more like SIV or SHIV in this aspect.
- Dr. Haigwood noted that some SHIV cases rebound after the antibody decays. She observed that, because SHIV has not been selected *in vivo*, SIV replication likely is more successful. Dr. Bar commented on the diversity of SHIVs and the range of viral fitness, adding that even the strongest SHIVs are less pathogenic than SIV, but SIV is more virulent than HIV.

DAY 2—TUESDAY, SEPTEMBER 24

SESSION 4: CURE RESEARCH IN ANIMAL MODELS

Session Chair: Nancy Haigwood, Ph.D., ONPRC

Gene and Immunotherapy for HIV Cure

Hans-Peter Kiem, M.D., Ph.D., University of Washington

Dr. Hans-Peter Kiem explained that the first clinical cases of transplant-based functional cure for HIV were accomplished with the transplantation of allogeneic HSCs that carried the CCR5 delta 32 mutation. These cases promote a proof of principle that HSCs lacking CCR5 (e.g., CCR5 delta 35 allele) combined with an allo-mediated anti-HIV effect (e.g., graft versus host or HIV) are critical components for an HIV cure. Dr. Kiem and his colleagues have now been modeling this approach in an autologous HSC transplant setting in NHPs. Dr. Kiem discussed two mechanisms for rendering hematopoietic cells resistant: CCR5 disruption and a C46 HIV fusion inhibitor. Both strategies can result in efficient protection of hematopoietic cells. Dr. Kiem further described that autologous HSCs can not only be rendered resistant to HIV infection with gene therapy and gene editing, but also be harnessed with an "allo" effect in the form of CAR-T cells against HIV-infected cells and the production of bNAb-secreting cells. He showed data that CAR-T cells generated from HSCs are functional and are able to target HIV/SHIV-infected cells. He also showed data that HSC can be modified to secrete bNAbs *in vivo*. Thus, the genetic modification of a patient's own HSCs could allow a less toxic and more feasible approach to develop an HIV cure based on cell and gene therapy

A Fully MHC-Matched Macaque Model of Allogeneic Stem Cell Transplantation
Jonah Sacha, Ph.D., Oregon Health & Science University

Dr. Jonah Sacha presented his work on the interplay between preclinical model biogenetics and cell transplantation. Allogeneic stem cell transplantation shows promise for treatment of HIV but is difficult to perform in a clinical setting. Dr. Sacha uses Mauritian cynomolgus macaques—an insular population with limited genetic diversity—as an animal model for cell transplantation. To ensure clinical relevance, Dr. Sacha worked with medical professionals to mirror the clinical protocols. He explained that this model demonstrates that the donor-versus-host allo response depletes the viral reservoir in SIV-infected animals; cells from the donor engraft successfully with full donor chimerism. The absence of CCR5 on donor cells, which is necessary for protection of the engrafting immune cells mediating the allo effect, remains a challenge. Dr. Sacha described ongoing efforts using Leronlimab, a drug that inhibits HIV by binding competitively to CCR5, to determine whether this treatment truly mimics the effects of CCR5 deficiency. Leronlimab is being tested in clinical trials and could be added to an existing therapy in efforts to develop a cure for HIV.

Evaluating Novel "Shock and Kill" Strategies in the NHP Model of HIV Infection
Maud Mavigner, Ph.D., Emory University School of Medicine

Dr. Maud Mavigner presented her research on "shock and kill" strategies (i.e., inducing HIV expression in latently infected cells to trigger cell death) in a rhesus macaque model. Dr. Mavigner explained that the concept has been demonstrated *in vitro* but is more challenging to perform *in vivo*. One promising shock-and-kill target is the SMAC, which has been used to promote apoptosis in tumor cells. Dr. Mavigner has demonstrated that the latency reversal activity of AZD5582 (a SMAC mimetic) is potentiated by CD8+ T cell depletion, suggesting that CD8+ T cells have a role in maintaining HIV and SIV latency. She stated that SMAC mimetics offer a promising strategy to reverse HIV latency. In an ongoing study, Dr. Mavigner is working to combine SMAC mimetics with clearance agents; she believes this strategy will enhance opportunities for HIV eradication.

Barcoded SIV and SHIV to Understand Reservoirs
Brandon Keele, Ph.D., NCI, NIH

Dr. Brandon Keele presented his work on tracking changes in SIV and SHIV lineages over time in rhesus and pigtail macaques. His strategy involves combining the strongest attributes of two types of viral stocks: swarms, which model a single patient and accumulate changes over time, and clonal stocks, which are more consistent between animals and are thus ideal for replicating published studies. Dr. Keele developed a synthetic swarm of virus—with a genetic barcode used to discriminate viral lineages—that mimics a diverse population. When a host is infected with a virus, diversity accumulates gradually over time; the barcodes, which replicate normally and maintain consistent frequencies, are useful for the characterization of such events as infection and reactivation. Dr. Keele stated that although this model does not represent an exact recapitulation of late ART in chronically infected individuals, it is a model of persistent virus over time that allows precise assessment of the dynamics of reservoir establishment, reservoir maintenance, reactivation, and subsequent rebound and possible reseeding of viral reservoir. He offered these barcodes to other investigators for use.

Engineering bNAb Expression *In Vivo*
Alejandro Balazs, Ph.D., Harvard University

Dr. Alejandro Balazs highlighted the utility of the humanized mouse model, explaining that he is interested in using vectors to deliver antibodies, an alternative to the traditional vaccine concept. He models these interventions in BLT humanized mice, which he defined as mice that display engraftment of human lymphocytes across tissues. The model has been used in the past for prevention studies and shows promise for therapeutic research. Dr. Balazs studied mice that were infected with different strains of HIV (i.e., REJO.c or JR-CSF) and treated with various bNAbs and found that the viruses accumulate mutations that define the underappreciated feature of antibody "escapability." Dr. Balazs suggested that escapability be considered for determination of antibody potency in conjunction with traditional potency and breadth metrics. He concluded by reiterating the benefits of the humanized mouse model (e.g., lower cost, the incorporation of human cells, the ability to perform complex experiments). He acknowledged drawbacks of the model (e.g., an incomplete immune system, low innate cell reconstitution and function, new HHS restrictions on fetal tissue), but theorized that the model will continue to improve in the future.

Panel Discussion Highlights

- When asked whether mutations observed *in vitro* might arise more slowly *in vivo* and how this would affect determination of the barcoded virus for early treatment, Dr. Keele explained that the system is being used to prevent undesirable viral bottlenecks. He reiterated the importance of defining barcode lineages over a long-term period.
- Several participants raised questions about the experimental design for the humanized mouse model, including questions about the mouse estrous cycle, microflora, and behavior.
- Participants discussed the benefits and drawbacks of humanized mouse models and their complementary role to studies performed in NHPs. Dr. Zack pointed out that Drs. Kiem and Mavigner have demonstrated studies consistent between humanized mouse and NHP models. He suggested that investigators work with mice initially to reduce study costs, although participants noted complications in tissue acquisition and their limitations for pediatric studies.
- Dr. Zack clarified that independently funded investigators often collaborate, allowing follow-up studies to mouse experiments. Participants emphasized the importance of individual expertise in comparing model species and suggested that an NIH Core Grant could serve to provide the services and expertise necessary for model organism studies. Dr. Haigwood pointed out that the NPRCs employ experts who could serve as potential collaborators for HIV studies.

SESSION 5: TOOLS AND TECHNOLOGIES
Session Chair: Francois Villinger, D.V.M., Emory University

NHP Genomics: Resources and Tools for Transcriptomics and Immune Repertoire Analysis
Steve Bosinger, Ph.D., Emory University

Dr. Steve Bosinger presented an overview of genomic resources available for rhesus macaques, tools for tracking B cell responses in NHPs, and the comparative genomics of NHPs relevant to HIV pathogenesis. He is developing bioinformatic tools and resources to track Ag-specific B cell response to vaccines in NHPs and humans, allowing paired immunoglobulin cloning, repertoire analysis, and estimation of somatic hypermutation rates. By sequencing one Indian rhesus macaque using long-read technology, Dr. Bosinger and his colleagues were able to double or triple the known alleles available at the

international ImMunoGeneTics information system® (IMGT®) and the National Center for Biotechnology Information, respectively. In another project, they sequenced the genome of the AIDS-resistant sooty mangabey monkey and identified unique mutations of the TLR4 gene that is conserved in multiple NHP natural SIV host species. Genomic references of the germline antibody gene loci require both further development to support HIV vaccine pre-clinical testing and proper application of the rhesus model for vaccines designed to elicit bNAbs. Dr. Bosinger and his colleagues currently are building a population-level Ig genomic reference database for rhesus macaques.

Marking Bone Marrow Stem Cells *In Vivo*
Cynthia E. Dunbar, M.D., Ph.D., National Heart, Lung, and Blood Institute, NIH

Dr. Cynthia Dunbar discussed clonal tracking of rhesus macaque HSCs and potential application to the study of HIV in humans. She uses genetic barcoding to investigate hematopoiesis at a clonal level in a rhesus autologous transplant model. Lessons from barcoding of hematopoietic stem or progenitor cells (HSPC) included the need to (1) utilize a sufficiently large and diverse library to achieve a high level of certainty that a barcode present in more than one cell comes from the same transduced precursor cell and (2) target a relatively low transduction efficiency so that most cells have only a single barcode. She was willing to share links to the code for the informatics utilized for barcode retrieval and analysis, which is available on GitHub. Dr. Dunbar used clonal tracking of the output of thousands of transplanted HSPC across lineages and over time. Notably, she discovered oligoclonally expanded clusters of clones contributing to CD16+ mature NK cells that waxed and waned over time, with no relationship to HSPC clonality patterns, suggesting peripheral NK response to an environmental cue. Dr. Dunbar and her colleagues examined the behavior of individual clones at the stem or progenitor cell level and found expansion of multipotent, myeloid, and B-biased clones with aging, as well as prolonged HSPC clonal segregation of local myeloid and B cell production in the marrow. Research questions that might be applicable to HIV research include whether stem and progenitor cell populations contribute equally to different lineages, determination of myeloid or lymphoid biases (which occurred with aging in NHP models), whether clonal expansion occurs with a specific clone predominating at different points in cell maturation, and whether specific clones expand only in a particular tissue area.

Spatial Imaging of HIV/SIV
Philip Santangelo, Ph.D., Emory University

Dr. Philip Santangelo described the use of PET CT for imaging SIV infection in macaques and developing probes for SHIV infections. He also discussed the application of this research to the imaging of HIV infection in humans, including lessons regarding image analysis and specificity. The approach used in his research, called ImmunoPET imaging, targets native viral molecules using antibody and antibody-based fragments labeled with an appropriate PET reporter. This imaging approach compared reasonably well to polymerase chain reaction testing. Investigators using PET imaging to study HIV in humans will need to consider antibody choice; bNAbs often do not bind to infected cells, so binders might be a better alternative. Another limitation of bNAbs is their long circulation time, which is undesirable for imaging. In addition, a radionuclide with a shorter half-life would be ideal. Limiting the circulation lifetime of the antibody, however, can limit sensitivity.

Quantification of PET signals is an important issue in molecular imaging, using such metrics as volume of interest and standard uptake value (SUV). Dr. Santangelo emphasized the need to evaluate the applicability of certain imaging metrics to molecular studies, because these metrics were developed for cancer and metabolic studies. He noted that total SUV within an organ might be a better metric than maximum and mean SUV signals, which provide less information on the reasons for changes in the

organs. He found that total SUV identifies the positive signal within an organ and provides a more accurate perspective; however, the approach is complex and labor intensive. Dr. Santangelo and his colleagues are working on automating the approach. Additionally, the team is attempting to assess PET CT specificity by linking PET CT to flow cytometry by combining PET-labeled probes with near-infrared fluorescence–labeled probes and comparing an *in vivo* probe with an *ex vivo* probe. This approach allows investigators to examine cells from individual infected tissues with high positive predictive values.

Fluorescently Marked Virus and bNAbs to Study Infected Cells
Thomas J. Hope, Ph.D., Northwestern University

Dr. Thomas Hope provided an overview on the use of imaging and fluorescently marked virus and bNAbs to study the site of SIV infection. Transmission and rebound both begin with single events; identification of the site of the initiation of infection/rebound can provide details of this process that could reveal new approaches to block these processes. Two approaches to tracking the site of viral infection are correlative bioluminescence and correlative PET CT (radioactive); Dr. Hope found the latter to be more sensitive because radioactivity easily passes through tissue. Labeling the virus both with both radioactivity and fluorescence provides an unbiased evaluation of virus distribution within a living macaque. The virus is labeled with a fluorescent protein, and imaging is used to see how the virus interacts with tissue. Dr. Hope used a photoactivated green fluorescent protein–labeled virus to overcome background interference. He reviewed his work using fluorescently tagged antibodies to examine the distribution and localization of intravenously instilled bNAbs. Studies of rebound (i.e., interruption of ART) demonstrated that the viral signal tends to return at the same general anatomical location in which it had disappeared at the onset of ART (usually in the gastrointestinal tract, female reproductive tract, and mesenteric lymph nodes, as well as the heart). Dr. Hope suggested that HIV- and SIV-related cardiovascular morbidity may result from infected cells in the heart causing myocarditis, rather than systemic inflammation. He noted that the methods he discussed can be used to explore this and other questions.

RNAscope Used for AIDS Research
Claire Deleage, Ph.D., NCI, NIH

Dr. Claire Deleage updated participants on a next-generation *in situ* hybridization technique that prevents nonspecific binding events so that signals can be amplified, and background noise can be suppressed to improve single-molecule visualization while maintaining tissue structure. This approach involves hybridizing pairs of small probes, which have high specificity and sensitivity. Dr. Deleage also described that using such short probes allows investigators to target different clades and chimeric viruses and to exclude different parts of the genome. These highly sensitive approaches allow the detection of viral RNA and DNA in the cells of hosts with an undetectable viral load. To phenotype cells harboring viral RNA and DNA, Dr. Deleage is combining the RNAscope and/or DNAscope assay with immunofluorescence staining for different cell markers. These approaches can have numerous applications in HIV/SIV research, such as examining early viral replication and dissemination, as well as viral persistence in NHPs. Dr. Deleage emphasized the importance of translating images to accurate quantifiable information that can be represented in charts and other forms of quantitative visualization—she collaborated with NIH computational science experts to apply deep learning technology on images and create a universal application for quantifying the number of virion and infected productive cells detected via RNAscope.

Repeated Biopsy Sample Collection Methods Used in AIDS Research
Jeremy Smedley, D.V.M., DACLAM, Oregon Health & Science University

Dr. Smedley presented an overview of modern techniques for biopsy sample collection in NHPs. He noted that the value of tissue sampling has been underscored throughout the workshop; specifically, both high throughput and minimal animal impact are critical to sampling success. He also emphasized the importance of controlling external factors—such as analgesics, anesthetics, and antibiotic treatment—to minimize variability among animals. He presented results from a study examining the effect of antibiotic treatment on the animal's microflora, noting that with his team's techniques, antibiotics generally are unnecessary. Dr. Smedley provided an overview of sampling techniques and procedures and discussed common issues in sampling, highlighting the importance of a balance between the amount of collected sample and the time interval between sampling points. He also noted techniques performed on biopsied samples, including viral load determination and RNA sequencing, and emphasized the feasibility—in terms of both cost and required skillset—of the collection techniques. He stated that these techniques are beneficial because they reduce variability and animal use, allowing researchers to obtain more data from a single individual. He further underscored the value of biopsy techniques for researchers. He noted that videos outlining several biopsy techniques (i.e., mesenteric lymph node and liver) have been published, and others (i.e., spleen) will be made available in the near future.

Panel Discussion Highlights

Participants offered no further discussion on this session.

IN-DEPTH DISCUSSION OF CURRENT AND FUTURE NEEDS—BREAKOUT GROUPS

Participants divided into breakout groups and discussed one area of future need.

FINAL DISCUSSION AND PRELIMINARY RECOMMENDATIONS

Group 1
Mario Roederer, Ph.D., NIAID

Question: What are the most critical improvements to existing models needed, and how can models and samples be shared more broadly?

Animal Acquisition and Funding

Group 1 suggested a central registry for used NHP animals to be made available to other investigators—animals ineligible for additional HIV research protocols can still be used for method development or behavioral studies. They also suggested supplementing base support for animal facilities to reduce the percentage of funding needed for animal acquisition.

Selection and Design of Virus-Challenge Stocks

Additionally, Group 1 discussed the need for new SHIVs for pathogenesis and cure studies that replicate more consistently, noting that having one stable SHIV that can be compared across studies would be more useful than developing more SHIVs. Researchers have begun making rhesus monoclonal antibodies against HIV for SHIV studies to complement existing monoclonal antibodies for SIV studies. Dr. Mario Roederer expressed optimism that NHPs can be used to model antibody interventions. He advocated for

more laboratories to make monoclonal antibodies against HIV and SIV in NHPs. For these experiments to be successful, the viruses must replicate persistently for at least 1 year.

Dr. Roederer advocated for researchers to report all viral loads, pathological data, and specific viral stock used, which would increase researchers' ability to compare and replicate studies. Group 1 noted that when NIAID provides stocks of challenge viruses, they require the data obtained from those viruses to be shared before the investigators receive additional stock. Dr. Roederer supported requirements that investigators adhere to at least a minimum standard of reporting information when publishing studies on challenge viruses. The scientific literature needs uniformity of data presentation and analysis. Participants noted that sequencing of challenge virus stock is available for free through the laboratory of Dr. Shelby O'Connor at the University of Wisconsin–Madison.

Discussion Highlights

- Dr. Lifson noted that challenge stocks are used to answer specific research questions, and meta-analysis is secondary.
- Use of the same viral stock, but with different dosages and in different laboratories, can produce different results.
- Participants supported a central web portal in which unique features of research methods and data are recorded transparently for the benefit of other investigators. The NIAID-sponsored ImmPort database can be used for sharing viral data.
- The NPRC is developing a primate clearinghouse called the Animal Locator to advertise and solicit research animals. Animal Locator is a secure system intended to be accessed by attending veterinarians. Researchers are interested in accepting recycled research animals from other institutions, but they often lack the necessary funds. Participants supported establishing a funding mechanism that would enable available animals to be transferred.

Group 2
Ann Chahroudi, M.D., Emory University School of Medicine

Question: What are the best models for each stage of the infection cycle (e.g., prevention, early infection, suppression/reactivation, aging with HIV) and different age groups (newborn, adolescent, adult, aging)?

Need for Further Characterization of Age Groups

Group 2's overall recommendations comprised increasing the size of breeding colonies, providing resources to support the study of all NHP age groups, and better characterization of normal function within each age group. For newborns, NHP models are superior to mice because of the sampling constraints inherent with smaller animals. The timing of transmission—*in utero* versus breastfeeding—influences model choice. Dr. Ann Chahroudi conveyed that researchers currently lack suitable characterization of the immune systems of infant macaques.

Group 2 observed that adolescent and aging NHPs are understudied. Juvenile NHPs could be used to study other STIs and drug abuse. Aging macaques are likely to have been used in many prior studies, so parity and hormones may influence results. Additionally, comorbidities common in older adult humans with HIV are difficult to study in macaques.

Opportunities for Iterative Research Between NHPs and Mice

Iterative studies between NHP and mouse models may be useful. Support for projects that enable the same mechanisms to be tested in both mice and NHPs could provide critical data. Group 2 suggested developing resources of humanized mice, as well as colonies of ART-suppressed macaques. Standard operating procedures for sample collection and preservation would assist uniformity of research methods. However, differences in microbiota must be accounted for between animals at different centers.

Discussion Highlights

Participants offered no further discussion on this topic.

Group 3
Francois Villinger, D.V.M., Emory University

Question: What new or underutilized models merit development (e.g., pigtail macaques, cynomolgus macaques, marmosets, or humanized mice or organoids)?

Development of Primatized Mouse Models

Dr. Francois Villinger expressed satisfaction with this conference's mouse model presentations, which characterized both the model's limitations and its possibilities. The limitations on human fetal tissue will negatively impact the availability of the humanized mouse model; mouse models created with cord blood are not as robust as those created using fetal tissue. This led to the discussion of potentially creating a primatized mouse model, which would require performing caesarian sections in the NHPs to obtain fetal tissue or cord blood.

Access to NHPs Remains a Challenge

Dr. Villinger indicated that increased support for macaque research and facilities is needed. Many NHP facilities currently have reached space limits for both breeding and research. Group 3 noted that a supply of cynomolgus macaques from Asia is available for researchers, but future availability is an area of concern. Academic investigators have had access to the Mauritian cynomolgus macaques required for transplant work, however, availability has diminished greatly as a result of competition from pharmaceutical buyers. The pigtail macaque model is excellent for disease progression, microbicide testing, and reproductive biology. Researchers have difficulty accessing enough animals for genetic modification studies; this limitation is particularly pronounced in rhesus macaques, which are seasonal breeders. Marmosets, which are currently available in limited supply, could potentially be used to examine neuromechanisms associated with HIV infection, including neuroinflammation, as the marmoset model is being used for neurological studies and they have a shorter lifespan compared to macaques. Initial studies characterizing the potential model would be necessary.

Discussion Highlights
Rhesus macaques are the preferred model for *Lactobacillus* colonization. Although rhesus macaques demonstrate regular menstrual bleeding throughout the year when exposed to controlled 12-hour day/night light cycles, their hormonal signatures differ during the non-breeding session and they do not become pregnant.

Group 4
Nancy Haigwood, Ph.D., ONPRC

Question: How can emerging technologies (e.g., imaging) be leveraged to facilitate HIV research in animal models?

Web Portal for Cross-Collaboration

Dr. Haigwood explained that large consortia funded by UM1 and P01 Research Program grant mechanisms have demonstrated success in sample sharing, idea sharing, and cross-fertilization; many external investigators are unaware of these trends. The group suggested that the NIH develop a web portal to make available to external investigators samples from ongoing UM1 studies in real time. Group 4 recommended that future UM1 applications be designed to ensure inclusion of early-stage investigators.

Support for Investigator Training and Sequencing

The group expressed support for current progress in viral stocks, including the generation of a panel of barcoded stocks. Researchers performing very large studies still have problems obtaining enough vials from centralized SHIV and SIV stocks. The centralized sequencing resource at the University of Wisconsin–Madison is helpful.

Group 4 also suggested that the NIH sponsor "hands-on/wet lab" training workshops involving travel to laboratories for instruction from experts in RNAscope, immunofluorescence antibody labeling, and laparoscopic procedures for obtaining biopsies from multiple tissues. Concerns regarding the budget limits for R21 grants were expressed, as the current limits are inadequate to support exploratory studies in NHPs. Researchers also expressed the benefit of more sequencing for all animals in the following areas: genome, microbiome, rectal, vaginal, B-cell receptors, and T-cell receptors. Genetic data would greatly enhance the utility of the animals for research.

Discussion Highlights

- Researchers have insufficient access to reagents to study NK cells in monkeys.
- Expanding the ClinicalTrials.gov system, in which investigators report their study design and results, to include animal studies would be a significant undertaking.
- Although ClinicalTrials.gov is a public site, a similar mechanism for animal studies would need to be hosted on a private system similar to the NIAID-sponsored ImmPort system.

Summary of Major Recommendations

- Develop a central registry to advertise and solicit previously studied and eligible NHPs for transfer between research facilities. The NPRC is developing a secure system for this purpose.
- Increase support for animal studies, animal acquisition, and facility infrastructure, exploring various mechanisms.
- Make a consistently replicating SHIV viral stock that could serve as a standard across studies.
- In both published literature and a proposed new web portal, researchers should report transparent details of study methods and data, including viral stock sequences and pathological data, to facilitate reproducibility.

- Address the need for greater supplies of several NHP species.
- Promote more research on age-specific groups of NHPs.
- Initiate a series of workshops to train researchers on specific techniques.

ADJOURNMENT

Dr. Haigwood thanked the participants for their contributions to the discussion. She expressed appreciation to OAR and ORIP for their logistical support in convening this meeting. Dr. Sheri Hild thanked everyone who assisted with planning and conducting the meeting. Dr. Haigwood adjourned the meeting.