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10–13 December 2019, Miami, Florida, USA

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Dear Colleagues

On behalf of the steering committee, I am pleased to welcome you to this Ninth International Workshop on HIV Persistence during Therapy.

Most of you are returning participants and for those who are joining us for the first time, welcome!

To remind you, this workshop covers subjects from basic science to clinical trials in humans and is aimed at both basic researchers and experienced clinicians as we discuss HIV reservoirs and eradication attempts.

I am *personally* disappointed that for the first time, the French National Agency for Research on AIDS (ANRS) is not present for the Workshop, however, I thank the NIH and NIAID for their continuous support.

Welcome to Miami and good work!

Alain Lafeuillade, PhD, Infectious disease private practice, La Valette du Var, France

Chairman of the Workshop

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Aims and objectives

The aim of this journal is to provide a specialist, open access forum and fast-track pathway to publish work in the rapidly developing field of virus eradication, particularly of HIV, HBV and HCV. The Journal has been set up especially for these and other viruses, including herpes and flu, in a context of new therapeutic strategies, as well as societal eradication of viral infections with preventive interventions.

Scope

The Journal not only publishes original research, but also provides an opportunity for opinions, reviews, case studies and comments on the published literature. It focuses on evidence-based medicine as the major thrust in the successful management of HIV and AIDS, HBV and HCV as well as includes relevant work for other viral infections. The Journal encompasses virological, immunological, epidemiological, modelling, pharmacological, pre-clinical and *in vitro*, as well as clinical, data including but not limited to drugs, immunotherapy and gene therapy. It will be an important source of information on the development of vaccine programmes and preventative measures aimed at virus eradication.

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CONTENTS

Abstracts of the 9th HIV Persistence During Therapy Workshop 10–13 December 2019, Miami, Florida, USA

Oral presentations may also be presented as poster presentations.

Session 1: Basic science of HIV latency

OP 1.1

Expression profiling of HIV latently-infected cells using nanostring and mass cytometry

H. Sperber[1,2], T. Ma[3], N.R. Roan[3], S.K. Pillai[1]

[1] Vitalant Research Institute, San Francisco, USA, [2] Free University of Berlin, Berlin, Germany, [3] Gladstone Institutes, San Francisco, USA

Background: The main barrier to an HIV cure is the latent HIV reservoir. Long-lived HIV latently-infected cells remain invisible to the host immune system and persist during antiretroviral therapy. In this study, we characterized latently-infected cells by implementing combined transcriptomic and proteomic profiling to identify unique expression signatures and reliable biomarkers that can be exploited to target and eliminate the latent reservoir.

Methods: Primary CD4+ T cells were purified from six healthy donors and were infected with a dual-reporter HIV construct that enables the isolation of HIV latently-infected and productively-infected cells by flow cytometry. The populations were then characterized using NanoString hybridization and fluorescence-based digital counting technology allowing for simultaneous detection of 770 mRNA and 30 protein targets, and mass cytometry (CyTOF), measuring 40 surface proteins. Target expression levels were compared between populations using false discovery rate (FDR<0.1), cellular pathways were analyzed using global significance scores, and upstream regulatory networks and causal relationships were deciphered using Ingenuity Pathway Analysis.

Results: The latent population displayed significant upregulation of CD73 protein and IL8 mRNA, and significant downregulation of CD39 mRNA compared to productively-infected cells and controls. Protein expression levels of T cell activation markers including CD25, PD-1, OX40, CD127, and GITR did not significantly differ between productively- and latently-infected cells, while ICOS, an inducible T cell costimulator, was significantly increased on latently-infected cells. The 'Pathogen defense' pathway was significantly suppressed in both HIV infected cell populations compared to uninfected controls. 'Antigen processing' was strongly suppressed in the latent population. Transcription factors FOXP1, FOXD1, and FOXJ1 were discovered as the top three master regulators in latent cells.

Conclusions: Our data suggest that HIV latently-infected cells exhibit distinct molecular features associated with an anergic and/or hypoxic T cell state, and may subvert antigen processing to remain immunologically invisible. FOXD1 and FOXJ1 likely repress HIV transcription in latently-infected cells through inhibition of NFkB and NFAT complexes. Our Results: warrant validation in vivo using clinical samples from ART-suppressed HIV-infected individuals, and mechanistic exploration ex vivo using targeted gene knockouts.

OP 1.2

A quantitative single cell, single molecule RNA-FISH+IF and single cell RNA-seq analysis reveals stochasticity of reactivation of latent provirus

G. Kalpana[1], R. Pathak[1], A. La Porte[1], E. Bock[1], C. Eliscovich[1], L. Martins[2], A. Spivac[2], U. Dixit[1], V. Planelles[2], R. Singer[1]

[1] Albert Einstein College of Medicine, New York, USA, [2] University of Utah School of Medicine, Salt Lake City, USA

Background: 'Shock and Kill' strategy to eliminate HIV-1 reservoirs requires complete reactivation of latent proviruses by Latency Reversing Agents (LRA), which is currently not possible. One reason for this variation is the stochasticity of proviral transcription. Studies using HIV-1 subviral clones with GFP markers have demonstrated stochasticity and identified intrinsic factors that play a role in proviral transcription. However, extrinsic factors that account for cell-to-cell variability of HIV-1 reactivation are not known.

Methods: We employed Stellaris based single molecule RNA-FISH combined with Immuno-fluorescence to study stochasticity in proviral reactivation. Using this highly sensitive method we tern SMIRA (Single-cell Single Molecule IF and RNA-FISH based Assay) and a method to quantitate single RNA molecules/cell (FISH-Quant), and a High Speed, high-Resolution Scanning (HSRS) to identify rare reactivated HIV-1^+ positive cells, we followed the kinetics of reactivation in T-cell models and ExVivo models of latency. Furthermore, we have applied 10x Genomics based single-cell RNA-seq (scRNA-seq) technology to sequence 5000 latent cells and identified factors that influence stochastic activation of HIV-1.

Results: We followed reactivation by 3 different clinically relevant LRAs using SMIRA and found that different LRAs exhibit different kinetics of reactivation and that any single drug did not sustain reactivation for a long period of time. We further found that the reactivation of latent cells is stochastic and that even in the absence of inducers ~5-10% of cells are active in latency models. Using scRNA-seq analysis, we identified a pool of cellular transcripts that are differentially regulated selectively in reactivated cells but not in non-reactivated cells. We found that these same transcripts were up-/down-regulated in induced cells via qRT-PCR, indicating that these transcripts are indeed correlated to HIV-1 reactivation. We further confirmed that the same factors were up-/down-regulated upon reactivation of latent cells in ExVivo models of latency.

Conclusions: Use of highly sensitive SMIRA revealed variability in the kinetics and stochasticity in reactivation of latent cells. Use of scRNA-seq revealed novel host factors responsible for stochastic proviral activation, which is likely to help design novel drugs to achieve more uniform activation or repression of proviruses.

OP 1.3

Single-cell transcriptome sequencing of latently-infected cells *ex vivo* using PCR-activated cell sorting (PACS)

E. Boritz[1], I. Clark[2,3], A. Abate[2], F. Quintana[3], S. Deeks[2], D. Douek[1]

[1] NIH, Bethesda, USA, [2] UCSF, San Francisco, USA, [3] Harvard, Boston, USA

Background: Rare CD4 T cells in blood and tissues that harbor replication-competent HIV during effective antiretroviral therapy (ART) represent a barrier to HIV cure. Defining the unique attributes of these cells could help enable novel HIV cure strategies, but highly pure ex vivo isolation of these cells – particularly those containing latent proviruses – has been technically challenging.

Methods: We modified PACS, an existing method for cell sorting based on nucleic acid detection within microfluidically-generated droplets, to allow the single-cell transcriptome sequencing of the HIV-infected 'latent reservoir.' Single cells from cell lines or peripheral blood were loaded at limiting dilution in water-in-oil droplets using a microfabricated polydimethylsiloxane (PDMS) device. Cells were lysed within droplets, allowing capture of genomic DNA and messenger RNA from each cell. Captured nucleic acids from millions of single cells were then subjected to HIV DNA detection, sorting of HIV-DNA+ droplets, and transcriptome recovery with the aid of two additional microfluidic devices. Sorted transcriptomes from HIV-DNA+ cells were Illumina sequenced.

Results: Experiments optimized RNA quality, microfluidic processing speed, compatibility of transcriptome recovery with HIV DNA detection, and water-in-oil droplet sorting accuracy. Single-cell capture of both mRNA and genomic DNA allowed separation of HIV DNA detection and transcriptome cDNA synthesis steps, reducing interference between the two. Optimization of HIV DNA detection conditions led to sensitive identification of single JLat cells that had been mixed at a 1:100 ratio with an uninfected mouse cell line. Transcriptome sequencing from sorted single cells of this mixture showed a low false-positive rate despite the large excess of uninfected cells. Optimized design and operation of all three microfluidic devices used in the workflow increased processing speed and accuracy sufficiently to enable hundreds of latently-infected cells to be sorted from PBMC of ART-treated individuals.

Conclusions: HIV-PACS allows detection, sorting, and single-cell transcriptome sequencing of cells that contain latent HIV genomes. This novel technology may yield important insights into the nature of the HIV-infected CD4 T cell reservoir during ART.

OP 1.4

Single cell analysis of *in vivo* HIV reservoir uncovers novel markers of latent cells

N. Roan[1], J. Neidleman[1,8], X. Luo[1], J. Frouard[1,8], F. Hsiao[1,8], G. Xie[1,8], V. Morcilla[2], KS. James[3], R. Hoh[4], M. Somsouk[5], P. Hunt[6], S. Deeks[4], N. Archin[3], S. Palmer[2], WC. Greene[1,7]

[1] Gladstone Institute of Virology and Immunology, San Francisco, CA, USA, [2] Centre for Virus Research, Westmead Institute for Medical Research, University of Sydney, Sydney, NSW, Australia, [3] Division of Infectious Diseases, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [4] Division of HIV, Infectious Diseases and Global Medicine, University of California San Francisco, San Francisco, CA, USA, [5] Department of Medicine, Division of Gastroenterology, San Francisco General Hospital and University of California, San Francisco, CA, USA, [6] Division of Experimental Medicine, University of California San Francisco, San Francisco, CA, USA, [7] Department of Medicine, University of California, University of California, San Francisco, CA, USA, [8] Department of Urology, University of California, San Francisco, CA, USA

Background: Direct phenotypic analysis of the in vivo latent HIV reservoir is complicated by the need to reactivate these cells ex vivo to identify them, which changes the phenotypes of the latent cells. We used CyTOF to quantitate the levels of 43 proteins on reactivated cells from ART-suppressed, HIV-infected individuals, and implemented a bioinformatics approach to trace each reactivated cell to its original latent state.

Methods: PBMCs (n=7), rectosigmoid biopsies (n=7), and lymph node aspirates (n=2) from treated individuals were phenotyped by CyTOF immediately after cell isolation, or stimulated with PMA/ionomycin or LRAs and then phenotyped. Reactivated cells were traced back to their original pre-stimulation state using the bioinformatics approach PP-SLIDE (Cavrois et al, Cell Reports 2017). Markers identified as preferentially expressed on latent cells were validated by conducting viral outgrowth assays and proviral sequencing on sorted cells.

Results: Latent cells were non-randomly distributed amongst memory CD4+ T cells. Markers preferentially expressed on latent cells included those that were shared between donors (PD1, CCR5, CD2, CD49d, Ox40) and donor-specific ones (CXCR5, TIGIT, CCR6, CD28, CD7). Markers differentially expressed between latent cells in blood vs. tissues, and between latent cells reactivatable by different stimulation methods, were identified. Analysis of longitudinal samples suggested the phenotype of latent cells is stable over time. Multiparameter sorting revealed that donor-shared surface markers identified by CyTOF markedly enriched for latent cells with replication-competent HIV: Tfh, already highly enriched for replication-competent HIV, was further enriched by 3 orders of magnitude using such markers. Viral sequencing revealed the enriched cells to be largely clonally expanded.

Conclusions: We have validated CyTOF phenotyping of reactivated latent cells paired with bioinformatics analysis by PP-SLIDE as an effective way to chart the in vivo blood and tissue reservoir. Our Results: demonstrate that 1) latent cells are not randomly distributed amongst memory CD4+ T cells, 2) the phenotypes of latent cells are stable over time, 3) LRAs target different latent cells than PMA/ionomycin, 4) there are shared as well as donor-specific surface markers of latent cells, and 5) sorting cells based on surface markers identified by CyTOF markedly enriches for clonally-expanded latent cells with replication-competent HIV.

OP 1.5

Quantifying the contribution of cellular proliferation to maintaining the HIV reservoir

A. Hill, G. Andrei, J. Gerold

Harvard University, Cambridge, MA, USA

Background: The HIV latent reservoir is extremely stable during antiretroviral therapy (ART), decaying with a half-life of ~ 4 years. In addition to the role of T cell longevity, recent characterization of viral integration sites and full-genome sequences has suggested that cellular proliferation also contributes to persistence. However, the rate of proliferation needed to explain the observed frequency of clones in the reservoir is unknown.

Methods: We developed a method to infer the underlying dynamics of the latent reservoir from clone size distributions in sampled cells. A set of stochastic mathematical models for latently infected cell turnover were created and fit to data using a Bayesian estimation procedure that accounts for the size of the reservoir before ART initiation, the time on ART, and the later sampling of cells. The algorithm was applied to data from integration site analysis and sequencing of replication-competent, intact, and total proviral DNA.

Results: Using integration site data and a simple homogeneous model of cell division and death, we estimated that on average latent cells divide around 10 times/yr [95% CI 5-20/yr], a much higher turnover than predicted by the slow decay. For individual patients, the best-estimated turnover rate varied by over ten-fold [2-30/yr]. This estimate was even higher for intact or replication competent cells (average ~20/yr). Comparing HIV and SIV clones, our model suggests that differences between the species can mainly be accounted for by different follow-up times and reservoir sizes. An augmented model allowing for rare, burst-like proliferation could explain the clone size distribution better than the simple model of homeostatic proliferation. We used simulation to confirm our inference method was unbiased and required ~500 samples from each patient to reduce uncertainty in turnover rate to +/–40% (95% CI).

Conclusions: Overall, these findings suggest that the latent reservoir turns over rapidly, and that therapy which marginally reduced proliferation could reduce dramatically reduce reservoir half-life, raising the potential for eradication with a few years of ART.

OP 1.6

Tyrosine kinase inhibition: the new front in HIV cure efforts

V. Planelles[1], M. Szaniawski[1], E. Williams[1], E. Innis[1], L. Martins[1], A. Spivak[1], J. Alcami[2], M. Coiras[2]

[1] University of Utah, Salt Lake City, USA, [2] Instituto de Salud Carlos III, Madrid, Spain

Background: The HIV-1 latent reservoir persists largely through the natural ability of memory T cells to homeostatically proliferate in response to gc-cytokines IL-7 and IL-15. In addition, HIV-1 also infects cells of the myeloid lineage, a cell type that can penetrate protected tissues including the central nervous system. Dasatinib and related tyrosine kinase inhibitors (TKI) that are FDA-approved for the treatment of chronic myeloid leukemia have several novel activities that can impact HIV-1 infection and the persistence of latent reservoirs. The first activity of dasatinib is the ability to block homeostatic proliferation. A second activity of dasatinib is its ability to block infection by HIV-1 by an unusual mechanism that involves dephosphorylation and activation of SAMHD1, a potent viral restriction factor.

Methods: To investigate the ability of TKIs to inhibit homeostatic proliferation of CD4+ T lymphocytes, we utilized a previously described model of latency based on primary cells, and induced proliferation via incubation with IL-2, IL-7 and IL-15. To study the inhibition of phosphorylation of SAMHD1, we cultured primary monocyte-derived macrophages and incubated them with TKI for 24hrs and then exposed them to HIV-1 Bal. SAMHD1 phosphorylation was evaluated by Western blot and flow cytometry.

Results: We demonstrate that TKIs block the homeostatic proliferation of latently infected T cells and thus prevent maintenance of the latent reservoir, and predict that this will result in accelerated decay over time. In vitro measurements demonstrate that various TKIs exhibit highly different inhibitory potency on lymphocyte proliferation.

We also observed that TKIs phenocopied the documented effects of types I and II IFNs on SAMHD1 dephosphorylation. The signaling pathway downstream of TKI is not associated with modulation of cyclin dependent kinases and is, therefore, different from that following IFN signalling.

The relative potencies of TKI (dasatinib, ponatinib, palbociclic, imatinib) in inhibiting cell proliferation did not correlate with the potencies at inhibiting phosphorylation of SAMHD1. Therefore, we speculate that the tyrosine kinase targets for both activities are likely different.

Conclusions: We conclude that TKI in vitro exhibit two different antiviral activities that are specific for HIV-1 and propose further development of these potentially exciting therapeutics.

PP 1.1

Remodeling of the core leads HIV-1 pre-integration complex into the nucleus of human lymphocytes

F. di Nunzio[1], G. Blanco-Rodriguez[1], A. Gazi[2], B. Monel[3], S. Frabetti[1], V. Scoca[1], O. Schwartz[4], J. Krijnse-Locker[2], P. Charneau[1]

[1] Department of Virology, VMV, Institut Pasteur, Paris, France, [2] Unit of Ultra-structural bio-imaging, Institut Pasteur, Paris, France, [3] Institut Pasteur, Paris, France, [4] Department of Virology, UVI, Institut Pasteur, Paris, France

Background: Retroviral replication proceeds through obligate integration of the viral DNA in the nucleus, essential for efficient viral replication. However, to be able to integrate into the host genome, the viral DNA must be led through the nuclear pore complex. Indirect evidences showed that HIV-1 capsid (CA) is the viral determinant for HIV-1 nuclear import. HIV-1 CA has been recently detected in the nucleus of dendritic or macrophage cells after infection but not in primary CD4+T cells, the latter are the main target cells for HIV-1. Therefore, the morphology of the CA protein associated to the functional PIC during its translocation is completely unknown. Our study aims to elucidate which is the morphology adopted by HIV-1 pre-integration complex (PIC) during the translocation into the nucleus. This step of the HIV-1 life cycle has never been previously elucidated because of the lack of appropriate technologies. Early steps of viral infection can be critical for the establishment of viral latency.

Methods: We applied immune gold labeling and correlative-light electron microscopy (CLEM) to detect the morphology of viral complexes. We also set up a new breakthrough method, called HIV-1 ANCHOR, to track the retrotranscribed DNA by live imaging or by CLEM.

Results: Our study provides a detailed view of the structural remodeling of the viral core prior, during and after HIV-1 nuclear entry. First, we observed that the uncoating is not completed before viral nuclear entry. Multiple CA proteins, forming 'pearl necklace shapes', can be translocated with the retrotranscribed HIV-1 DNA inside the nucleus. These Results: indicate that the viral core remodels in partial cores (continue or discontinue structures) during the nuclear passage through the pore. Importantly, our data are the first proof of concept of the possibility to directly target and follow the fate in live cells of HIV-1 DNA during and after nuclear entry.

Conclusions: This study gives a new outlook of how the viral CA remodels to allow the nuclear translocation of HIV-1 PIC. We are now able to live-track the viral DNA in natural HIV-1 target cells. This innovative approach could provide new insights into the mechanisms underlying viral persistence.

PP 1.2

Combination of quadruplex qPCR and next-generation sequencing for qualitative and quantitative analysis of the HIV-1 latent reservoir

C. Gaebler

Rockefeller University, New York, USA

Background: HIV-1 infection requires lifelong therapy with antiretroviral drugs due to the existence of a latent reservoir of transcriptionally inactive integrated proviruses. The goal of HIV-1 cure research is to eliminate or functionally silence this reservoir. To this end, there are numerous ongoing studies to evaluate immunological approaches, including monoclonal antibody therapies. Evaluating the Results: of these studies requires sensitive and specific measures of the reservoir.

Methods: We describe a relatively high-throughput combined quantitative PCR (qPCR) and next-generation sequencing method. Four different qPCR probes covering the packaging signal (PS), group-specific antigen (gag), polymerase (pol), and envelope (env) are combined in a single multiplex reaction to detect the HIV-1 genome in limiting dilution samples followed by sequence verification of individual reactions that are positive for combinations of any two of the four probes (Q4PCR). We compare the new method to quantitative and qualitative viral outgrowth assays (Q^2VOAs) and near full-length (NFL) sequencing on paired peripheral blood samples obtained at two time points from the same six individuals enrolled in a clinical trial that involved analytical treatment interruption after infusion of a combination of two broadly neutralizing monoclonal antibodies.

Results: All intact viruses in the six individuals analyzed and 99% of the intact clade B viral sequences in the Los Alamos database were positive for any combination of two of the four probes in the Q4PCR reaction. The advantage of Q4PCR is that the method is relatively rapid, scalable, and both sensitive and specific. Bar coding and next-generation sequencing facilitates the analysis of large numbers of samples simultaneously and enables definitive identification of intact proviruses. In addition, the sequence data provide information on the clonal structure of the latent reservoir and on the nature of the defective proviruses.

Conclusions: The combination of four-probe qPCR and next-generation sequencing is a highly sensitive and specific method for measuring intact proviruses in the HIV-1 latent reservoir.

PP 1.3

Single cell analysis reveals molecular signatures of HIV latency in primary cell models

S. Telwatte[1,2], M. Montano[3], R. Resop[4], E. Battivelli[5], S. Morón-López[1], E. Verdin[5], W. Greene[3], A. Bosque[4], J. Wong[2], S. Yukl[2]

[1] University of California, San Francisco, USA, [2] Francisco VA Health Care System, San Francisco, USA, [3] Gladstone Institutes, San Francisco, USA, [4] George Washington University, Washington, USA, [5] Buck Institute for Research and Aging, San Francisco, USA

Background: Primary cell models have greatly advanced our understanding of HIV latency. However, it is unclear what mechanisms underlie latency in these primary cell models. We hypothesized that molecular signatures can distinguish uninfected, latently- and productively-infected populations in these models.

Methods: We assessed 4 primary cell models [blood CD4T cells: models from labs of Eric Verdin, Alberto Bosque, and Warner Greene; tissue(tonsillar) CD4T cells: model from Warner Greene]. Single cells from each model (2 donors) were FACS-sorted into 96-well plates and multiplex RT-qPCR (BiomarkHD) was used to quantify 88 human RNAs previously implicated in HIV infection/latency and 8 HIV targets (5'LTR, Gag, Pol, Nef, MS Tat-Rev, U3-PolyA, and the IPDA assays for Envand Gag). We compared HIV-unexposed, HIV-exposed but uninfected, and latently- +/− productively-infected populations from each model to identify genes with ≥2-fold difference in median expression levels and P<0.05(*) or FDR-corrected P<0.05(**).

Results: As expected, multiple HIV targets(**) distinguished uninfected, latently-infected, and productively-infected cells. Each model differed in the cellular factors that distinguished populations, with some differences between donors. Compared to HIV-unexposed cells, latently-infected cells from the Verdin model showed higher expression of CXCR4(**), POL2RA(**), APOBEC3G(*), and STING(*) and lower expression of PRMT6(*), while latent cells from the Bosque model expressed higher levels of CGAS(**), and latent tonsil cells from the Greene model showed higher expression of CDK7, PBAF, RIG-I, and MDA5 (*for all). Compared to HIV-exposed but uninfected cells, latently-infected cells showed: 1) less CCR5(*), CD38(*), and NF-KBIA(*), but higher CD25(*) expression in the Verdin model; 2) less Cyclin L2(*) and more BCL6(*) in the Bosque model; and 3) no difference (except HIV targets[**]) in blood cells from the Greene model. Relative to productively-infected cells, latently-infected cells upregulated CTLA-4, BCL-11B, NFATC1, CDK7, HTATSF1, PAF-1, and PBAF expression (**for all) in the Verdin model, and exhibited lower expression of CD28, CTLA-4, PD-1, BCL-6, BCL-11B, FAS, Sp1, POLR2A, CREBBP, G9a, STAT1, and IRF9 (**for all) in the Greene tonsil model.

Conclusions: Our analysis shows multiple cellular factors that distinguish latently-infected from uninfected and productively-infected cells, that may provide a molecular signature necessary to identify this population in vivo

PP 1.4

Evaluation of IAP/SMAC mimetics as latency reversal agents in primary cells and cytokine induction in *in vivo* models predictive of cytokine release

B. Howell[1], W. Shipe[1], G. Adam[1], S. Quan[1], L. Li[2], C.N.A. Sim[2], R. Dunham[3], D. Margolis[4], B. Henry[2], D. Hazuda[1]

[1] Merck & Co., Inc., West Point, USA, [2] MSD Singapore, Singapore, [3] Viiv, Chapel Hill, USA, [4] UNC, Chapel Hill, USA

Background: HIV persists in latent reservoirs as transcriptionally silent provirus, presenting an obstacle for cure. Considering multiple mechanisms maintain HIV latency, several strategies aimed at inducing viral expression and triggering apoptosis or immune-mediated clearance of infected cells are actively being pursued. Inhibitors of apoptosis proteins (IAPs) / second mitochondrial-derived activation of caspases (SMAC) mimetics can reverse latency and selectively induce apoptosis in HIV+ CD4 T cells through activation of non-canonical NF-kB signaling, inhibition of cell survival signals, and cytokine production. We assessed the HIV reactivation of IAPs/SMAC mimetics +/− HDAC inhibition in vitro employing a primary latency model in CD4+ T-cells, cytokine production in human PBMCs, and compared in vivo cytokine release in C57BL/6J and the HuNOG-EXL humanized mouse model induction.

Methods: HIV reactivation +/− vorinostat was measured in a primary CD4+ T cell model of latency. Measurement of cytokines (IFN-r, IL-1B, IL-2, Il-4, IL-8, IL-10, IL-12p70, IL-13 and TNF-a) was performed in PBMCs using MesoScale Discovery Proinfammatory panel 1. C57BL/6J mice were used to assess plasma cytokine expression following SMAC mimetic administration. HuNOG-EXL mouse (Taconic) was validated using lipopolysaccharide (LPS) and anti-CD28 antibody and used to predict cytokine release potential of candidate therapeutic agents.

Results: HIV latency reversal was observed with IAP/SMAC mimetics alone and increased in combination of vorinostat. AZD-5582 showed robust IL6 elevation and dose-dependent broad cytokine increases in uninfected PBMCs, while other SMAC mimetics displayed minimal cytokine elevation LCL-161 administration assessed in C57BL/6J and HuNOG-EXL mouse model suggests dose- and time-dependent increases in proinflammatory cytokines.

Conclusions: IAPs/SMAC mimetics represent an emerging approach for HIV latency reversal and cure. Here, we demonstrate enhanced latency reversal with vorinostat and highlight the use of in vitro and in vivo model systems for deeper characterization of emerging candidate therapeutics with potential cytokine liabilities.

Conflict of interest: Howell, Shipe, Adam, Quan, and Hazuda are employees of Merck & Co., Inc.

Li, Sim, and Henry are employees of MSD.

Dunham is employee of Viiv.

PP 1.5

Live track of HIV genome in the nuclear space

F. Di Nunzio[1], V. Scoca[1], G. Blanco-Rodriguez[1], D. Ershov[2], J.Y. Tinevez[2]

[1] Department of Virology, Institut Pasteur, Paris, France, [2] Image Analysis Hub/ C2RT, Institut Pasteur, Paris, France

Background: Viral genome and host nuclear compartment interplay can determine virus- host coexistence prompting the release of new viral progeny. HIV-1 needs to release high levels of newborn viruses within few days from infection. Viral integration within active genes is usually favorable for efficient transcription, potentially explaining the targeting preference. However, HIV-1 can also coexist for prolonged periods as chimera in the genome of infected patients as latent virus. In particular viral latency can be determined by the nuclear environment surrounding viral DNA. However, the mechanisms underlying the establishment of latency are under investigation. The state of nuclear viral forms and the nuclear environment can determine the evolution of HIV infection. Therefore, characterizing the fate of viral genomes is crucial to understand the viral life cycle. On this study we adapted an ex vivo DNA tagging technology, called ANCHOR, to label the retrotranscribed DNA of HIV. This is a bipartite system derived from a bacterial ParABS chromosome segregation machinery. This breakthrough approach is also compatible with RNA live imaging MS2 tool.

Methods: To perform live imaging experiments, we used HIV genome that contains two sequences, one targeted by ParB modified (protein binding the DNA) and the other by MCP which binds the viral RNA. Both labeling viral genome proteins are stably expressed using lentiviral vectors.

Results: We specifically tracked HIV-1 DNA integrated forms as well as nuclear viral RNA transcription foci. HIV-1 ANCHOR efficiently

visualizes viral proviruses, as confirmed by AluPCR. The tracking of viral DNA is highly specific as corroborated by the absence of nuclear signals in presence of drugs that block nuclear import or reverse transcription. Integrated or extrachromosomal can be directly tracked in infected cells. Results: showed a different diffusion coefficient between proviruses and episomal viral forms. Surprisingly, episomal forms consist of two populations that behave with a different diffusion coefficient.

Conclusions: Our study in live cells permits to follow the dynamics of HIV genome and transcription in spatio-temporal mode. Future experiments could elucidate mechanisms and factors involved in the transition from a latent virus to an active virus and vice versa in primary CD4+ T cells.

PP 1.6

Genome-wide RNAi screen identifies MAPK-RPK required for HIV-1 proviral silencing in non-T cell reservoir cell-line model

H. Takeuchi[1]*, T. Ishida[2]*, Y. Satou[3,4]*, J. Gohda[2], H. Kitamura[1], S. Gan[1], K. Takahashi[1], S. Yamaoka[1]

*[1] Department of Molecular Virology, Tokyo Medical and Dental University, Tokyo, Japan, [2] Research Center for Asian Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan, [3] Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan, [4] International Research Center for Medical Sciences, Kumamoto University, Kumamoto, Japan, *authors contributed equally*

Background: Currently, the establishment of HIV-1 persistent infection is still a major barrier to complete viral eradication, which has remained elusive because latently infected reservoirs can evade host antiviral immune responses as well as combination antiretroviral therapy. There is therefore still an unmet need to develop new anti-HIV compounds which efficiently reactivate latent HIV-1 provirus. Recent studies indicate that the responsiveness of the latent provirus to each latency-reversing agent (LRA) could be dependent on the reservoir cell type. It is therefore necessary for us to deepen our understanding of molecular mechanisms of HIV-1 latency.

Methods: To identify host cell factor involved in HIV-1 latency, we employed a genome-wide RNAi screen in newly established latently infected model monocyte-linage cell clone, THP-1 NanoLuc #225 (#225 clone), capable of expressing NanoLuc (Nluc) protein in the *nef* region of HIV-1 provirus and found that Nluc activity in MAP kinase-related protein kinase (MAPK-RPK)-depleted #225 clone was markedly enhanced. To investigate whether the shRNA has an off-target effect on HIV-1 activation or not, we next established MAPK-RPK-depleted #225 clone with different MAPK-RPK-targeting short hairpin RNAs (shRNAs) and analyzed Nluc activities from the cell lysates. To examine if MAPK-RPK depletion affects Tat-dependent HIV-1 activation, we transduced HIV-1 Tat-targeting shRNA into MAPK-RPK depleted #225 clone and analyzed Nluc activities from the cell lysates.

Results: Nluc reporter activity in #225 clone was substantially enhanced by MAPK-RPK depletion, suggesting the possible involvement of MAPK-RPK in HIV-1 latency. Enhanced Nluc activities by MAPK-RPK depletion with three different MAPK-RPK-targeting shRNAs was dependent on its level of expression, reducing the likelihood of an off-target effect of the shRNA. HIV-1 Tat depletion in MAPK-RPK-depleted #225 clone markedly reduced HIV-1 transcription to the same level as control non-targeting shRNA transduced #225 clone, suggesting the possible involvement of MAPK-RPK in HIV-1 transcriptional elongation. Forced-expression with wild type but not catalytically inactive MAPK-RPK significantly reduced HIV-1 transcription, suggesting that the kinase activity of MAPK-RPK is required for maintaining HIV-1 latency.

Conclusions: Our Results: indicate the essential role of MAPK-RPK for maintaining HIV-1 latency and this may encourage development of a new class of LRA.

PP 1.7

Intact HIV genomes are enriched in memory T cells with short half-lives

V. Morcilla[1], C. Bacchus-Souffan[2], T. Schlub[3], M. Fitch[4], R. Hoh[5], S. Deeks[5], M. Hellerstein[4], J. Mccune[6], P. Hunt[2], S. Palmer[1]

[1] Centre for Virus Research, Westmead Institute of Medical Research, University of Sydney, Westmead, Australia, [2] Division of Experimental Medicine, Department of Medicine, University of California San Francisco, San Francisco, USA, [3] Sydney School of Public Health, Faculty of Medicine and Health, University of Sydney, Australia, [4] Department of Nutritional Sciences and Toxicology, University of California, Berkeley, USA, [5] Division of HIV, Infectious Diseases and Global Medicine, Department of Medicine, Zuckerberg San Francisco General Hospital, University of California San Francisco, San Francisco, USA, [6] Global Health Innovative Technology Solutions/HIV Frontiers, Bill & Melinda Gates Foundation, Seattle, USA

Background: Future HIV curative therapies require a thorough understanding of the distribution of genetically intact HIV within T-cell subsets during short-term antiretroviral therapy (ART) and the cellular mechanisms which maintain this reservoir. Therefore, we genetically characterized HIV genomes within T-cell subsets from participants on <4 years of therapy.

Methods: Participants were treated for <4 years either within 5 months (early, n=4) or after 7 months (late, n=3) of HIV infection. Near full-length proviral sequences were obtained from peripheral blood naïve (NV), stem-cell memory (SCM), central memory (CM), transitional memory (TM), effector memory (EM), and terminally differentiated (TD) CD4+T-cells. Clusters of ≥2 proviral sequences which were 100% genetically identical and indicative of host cell proliferation were identified. Cellular half-lives were measured by in vivo incorporation of deuterium into genomic DNA within these cell subsets.

Results: A total of 893 sequences were isolated; 585 and 308 from the early and late ART participants respectively. From these 893 sequences, 57 were considered intact (6.4%); 13 and 44 respectively from the early and late ART groups. The proportion of intact sequences across the T-cell subsets was different (p=0.03). In the late ART group, the intact sequences were concentrated in cells with shorter half-lives such as TM ($6/10^6$ TM-cells; median half-life: 95 days) and EM cells ($25/10^6$ EM-cells; median half-life: 82 days) compared to other subsets with longer half-lives (median half-lives: 162-1107 days for NV, SCM, CM, and TD cells). For the early and late ART groups, a correlation was found where cells with shorter half-lives contained more intact proviruses (p=0.03). The levels of identical sequences contributing to a cluster were highest within EM and TD in all participants (p<0.001).

Conclusions: The distribution of HIV genomes across T-cell subsets during short-term therapy after both early and late ART suggests that a short cellular half-life could be a predictor of a higher frequency of intact proviruses. Both TD and EM cell subsets were marked by clusters of identical HIV genomes reflecting cellular proliferation. This indicates that specific cellular mechanisms such as a short half-life and greater proliferative potential, characteristics of EM T-cells, contribute to the maintenance of intact HIV.

PP 1.8

HBV-related inflammation is linked to the level of genetically intact HIV proviruses

X. Wang[1,2], J.M. Zerbato[3], A. Avihingsanon[4], A. Rhodes[3], J. Audsley[3], K. Singh[3], W. Zhao[3], M. Crane[3], S. Lewin[3], S. Palmer[3]

[1] Centre for Virus Research, Westmead Institute for Medical Research, Westmead, Australia, [2] Sydney Medical School, University of Sydney, Sydney, Australia, [3] Peter Doherty Institute for Infection and Immunity, University of Melbourne and Royal Melbourne Hospital, Melbourne, Australia, [4] 4HIV-NAT, Thai Red Cross AIDS Research Center, Bangkok, Thailand

Background: Hepatitis B virus (HBV) coinfection increases overall and liver-related mortality in people living with HIV, even with the availability of HBV-active ART. HIV can persist in individuals in both defective and intact forms and both can contribute to persistent inflammation. We assessed the relationship between HIV proviral genomes and markers of inflammation in people living with HIV-HBV coinfection.

Methods: HIV-HBV coinfected and HIV monoinfected participants, naïve to ART, were recruited in Bangkok, Thailand as part of a prospective observational cohort study. HIV subtype AE proviruses were sequenced from peripheral blood (PB) CD4+ T-cells using full-length individual proviral sequencing, covering 92% of the genome. Circulating markers of inflammation and microbial translocation were quantified by ELISA and bead arrays. Spearmans rank correlations tests were performed to determine associations.

Results: 1008 and 222 HIV proviruses were sequenced from 18 HIV-HBV coinfected and 6 HIV monoinfected individuals respectively. The coinfected cohort had a significantly higher HIV viremia (p=0.03) and lower CD4+ T-cell count (p=0.007) than the monoinfected. A strong trend towards more intact proviruses (22-1000 copies/106 cells, p=0.055) was observed in the coinfected individuals. For the HIV-HBV cohort, the levels of soluble CD14 (sCD14), LPS and CXCL10 in the blood, markers of immune activation and/or inflammation, were significantly correlated with the frequency of intact HIV proviruses (p<0.01, p=0.04, p<0.01 respectively). sCD14 and CXCL10 were also correlated with the genetic diversity of the intact proviruses (p=0.03, for both). AST levels in blood, a marker of liver inflammation, and HIV DNA levels in the liver were also significantly correlated with the frequency of intact HIV proviruses in PB CD4+ T-cells (p=0.04, p=0.05 respectively). However, intact proviruses alone did not correlate with the number of PB CD4+ T-cells (p=0.2) but the inclusion of defective forms revealed a significant correlation with PB CD4+ T-cells (p=0.03).

Conclusions: During HIV-HBV coinfection, the levels of PB CD4+ T-cells may be influenced by the amount of intact and defective proviruses they contain. However, the frequency and genetic diversity of the intact proviruses within blood-derived cells from the HIV-HBV coinfected individuals appears to be linked to inflammation and liver damage.

PP 1.9

Intact proviruses from naive and effector memory T-cells match persistent viremia

K. Fisher[1], B. Hiener[1], T.E. Schlub[2], E. Lee[1], J.M. Milush[3], R. Hoh[3], R. Fromentin[4], N. Chomont[4], S.G. Deeks[3], S. Palmer[1]

[1] Westmead Institute for Medical Research, Westmead, Australia, [2] University of Sydney, Sydney, Australia, [3] University of California San Francisco, San Francisco, USA, [4] Université de Montréal, Montreal, Canada

Background: Genetically intact, and potentially replication-competent, proviruses are a likely source for viremia during antiretroviral therapy (ART). Identifying the CD4+T-cell subsets that harbour these proviruses within different anatomic sites is important for future eradication strategies.

Methods: Near full-length proviral sequences were obtained from naïve (NV), central (CM), transitional (TM) and effector memory (EM) CD4+T-cells (sorted based on their expression of CD45RA, CD27 and CCR7), which were isolated from both the peripheral blood (PB, 13 participants) and lymph nodes (paired LN, 5 participants), using the Full-Length Individual Proviral Sequencing Assay (FLIPS). Proviral sequences were identified as genetically intact or defective. Genetically intact proviruses from 10 participants were compared to on-therapy plasma RNA (p6-RT region obtained by single-genome sequencing (SGS)).

Results: We sequenced 1913 proviruses, and genetically intact proviruses were found in all cell subsets except LNEM (n=3). We found that the infection frequency of genetically intact proviruses differed across the subsets in both PB and LN (P<0.001). In PB, the order of intact genomes was found to be EM>TM/NV>CM (all P<0.02), while in the LN the trend was NV>TM>CM>EM, with evidence for NV>CM (P=0.01). All 22 intact LN sequences were genetically unique. For the subsets that had more than 10 genetically intact DNA sequences (PBEM, PBNV and LNNV), we compared the genetically intact proviruses obtained by FLIPS to the on-therapy plasma RNA p6-RT sequences from SGS. PBEM had the highest frequency of genetically intact DNA sequences matching 100% to the on-therapy RNA sequences (13/23, 57%). This was followed by PBNV, with 6/19 (32%) DNA sequences matching RNA, and LNNV, with 3/16 (19%) DNA sequences matching RNA.

Conclusions: The distribution of genetically intact proviruses differs between PB and LN. For the five participants with paired PB and LN cells available, NV cells had the highest frequency of intact proviruses in LN. In PB, however, the highest levels of intact genomes were found in EM cells. PBEM, PBNV and LNNV also had a high frequency of genetically intact proviruses matching to on-therapy plasma RNA p6-RT sequences, suggesting that the intact proviruses within these T-cell subsets from different anatomic sites may contribute to ongoing viremia during ART.

PP 1.10

Global mapping of the macrophage transcriptome upon CCL2 neutralisation reveals an association between activation of innate immune pathways and HIV-1 restriction

D.A. Covino[1], I Fantuzzi[1], J. Lu[2], M.V. Chiantore[3], G. Fiorucci[4], C. Purificato[1], L. Catapano[1], C.M. Galluzzo[1], R. Amici[1], M. Pellegrini[2]

[1] National Center for Global Health, Istituto Superiore di Sanità, Rome, Italy, [2] Department of Molecular, Cell, and Developmental Biology, University of California Los Angeles, Los Angeles, USA, [3] Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy, [4] Institute of Molecular Biology and Pathology, CNR, Rome, Italy

Background: Residual viremia and low-grade chronic inflammation in cART-treated subjects are nowadays considered the main challenges to achieve a cure. The CCL2/CCR2 axis plays key roles in chronic inflammation in these patients. We found that CCL2 blocking in macrophages restricts HIV-1 replication by inhibiting viral DNA accumulation. This effect was independent of SAMHD1 and was associated with induction of APOBEC3A expression, which was also increased in HIV-1-infected subjects treated with the CCR5/CCR2 inhibitor cenicriviroc. This study aimed at a deeper characterization of the cellular factors/pathways modulated by CCL2 blocking in macrophages and potentially involved in the restriction of HIV-1 replication.

Methods: Analysis of mRNA and miRNA expression profiling was performed on monocyte-derived macrophages treated with anti-CCL2 or control antibodies and infected with HIV-1BaL. Genes with FC>2 and adjusted p value <0.1 and miRNAs with FC>2 and p value < 0.05 were classified as differentially expressed. Functional analysis was done using DAVID and TRRUST. The differential expression profile of some miRNAs/transcripts was confirmed by qPCR.

Results: Functional annotation clustering revealed two clusters of up-regulated genes which included genes related to antiviral defense/innate immunity (cluster 1, enrichment score 11.5, 83 genes) and immune response/inflammatory response (cluster 2, enrichment score 9.5, 113 genes). The clusters comprised several interferon-stimulated genes, such as DDIT4, GBP1, IFI44L, IFIT3, IRF1, IRF7, ISG20, OAS3, OASL, and the restriction factor coding genes APOBEC3A, EIF2AK2, GBP5, HERC5, IFITM1, IFITM3, ISG15, MX1, RSAD2, and TRIM25. The genes included in the clusters were enriched for RELA and NFKB1 targets, indicating the activation of the canonical NF-kB pathway following CCL2 blocking, and a regulatory network involving NFKB1, NFKB1 targets and miR-155 was identified and validated. Furthermore, CCL2 blocking restored the activation of host defense genes in HIV-infected macrophages, associated with a potent inhibition of viral transcript expression.

Conclusions: Overall, these data highlight an association between activation of innate immune pathways and inhibition of HIV-1 gene expression upon CCL2 blocking in macrophages and identify CCL2 as an endogenous factor contributing to the deficient macrophage

response to HIV-1. Targeting this chemokine may thus represent an effective therapeutic strategy to strengthen host innate immunity and restrict HIV-1 replication.

PP 1.11

HIV-1 replication is metabolically regulated in an *ex vivo* human tonsil histoculture model of infection

R. Furler, K. Newcombe, D. Nixon

Weill Cornell Medicine, New York, USA

Background: Productive HIV-1 replication occurs when an infected CD4+ T cell is in an activated state. T cell activation reorganizes the cytoskeleton, upregulates membrane expression of nutrient transporters, and redirects cellular metabolism to anabolic pathways that increase nucleic acid, protein, and lipid production. Aerobic glycolysis ensues and there is an increase in oxidative phosphorylation to meet the energy needs of building new biomolecules. When a T cell transitions into a memory cell, these biochemical pathways are downregulated as the T cell migrates through the lymphatics and blood until encountering another antigen. During this transition, the HIV-1 provirus becomes latent. Since reversing latency is a major goal of many in the field, we will approach latency initiation and reversal through a metabolic lens.

Methods: Several in vitro models use CD4+ T cell lines, primary CD4+ T cell subsets, or PBMC; however, these lack the 3-dimensional structure of lymphoid tissues, where HIV-1 replication primarily occurs. We use a previously optimized *ex vivo* tonsil histoculture model of HIV-1 infection. This uses small pieces of 3-dimensional lymphoid tissue that can sustain HIV-1 replication for several weeks. To measure the effects of metabolism on HIV-1 replication kinetics, we infected the histocultures with a CCR5-tropic HIV-1 isolate, ADA, with or without inhibitors of glycolysis and oxidative phosphorylation (2-DG and Oligomycin respectively). HIV-1 virions in the supernatant were measured over time using a HIV-1 Gag p24 ELISA.

Results: HIV-1 replication kinetics was measured in the ex vivo histoculture models every 3-4 days for at least 3 weeks. Four tonsil sections (~1 mm3each) were placed on a collagen support in each well of a 6-well plate. Each condition had at least two replicate wells. Supernatants from each well was measured in duplicate for HIV-1 Gag p24 by ELISA. Both metabolic inhibitors, 2-DG and Oligomycin, significantly decreased HIV-1 replication compared to untreated in this system.

Conclusions: Following T cell activation, increased aerobic glycolysis and oxidative phosphorylation occurs to meet the biomolecule and energy needs of a growing and/or proliferating cell. Inhibiting these pathways in our ex vivo histoculture model significantly reduced HIV-1 replication.

PP 1.12

Histone deacetylase inhibitors induce transcription of unspliced but not multiply spliced HIV-1 RNA from proviral genomes during latency reversal, affecting antigen presentation and detection by CD8+ T cells

T. Mota[1], C. Mccann[1], S.H. Huang[1], M. Dean[1], R. Yanqin[1], R. Thomas[2], K. Colin[3], H. David[4], S. Jeffery[5], J. Brad[5]

[1] Weill Cornell Medicine, New York, USA, [2] George Washington University, Washington, USA, [3] Maple Leaf Clinic, Toronto, Canada, [4] Whitman-Walker Health, Washington, USA, [5] NantBioScience Inc./NantKwest LLC, Culver City, USA

Background: A successful latency reversing agent (LRA) must induce viral transcription and expose HIV reservoirs to clearance by immune effectors, such as CD8+ cytotoxic T lymphocytes (CTLs). Histone deacetylase inhibitors (HDACi) tested as LRAs in clinical trials induced transcription of unspliced (US) HIV RNA, but did not reduce frequencies of infected cells. In other research fields, HDACi are used to inhibit splicing to restore wildtype protein, or to impair CD8+ T-cell function. To investigate these activities, we compare a new class-I-selective HDACi, nanatinostat, to romidepsin and the PKC agonist bryostatin.

Methods: We measured the abilities of LRAs to induce viral transcription and splicing in ex vivo CD4+ T-cells from ARV-treated individuals. HIV-specific CTL clones specific for either an US (Gag) or spliced (Nef) product were used as biosensors to detect LRA-driven antigen presentation in a primary-cell latency model. Viral inhibition assays (VIAs), were used to test the effects of LRAs on the abilities of primary CD8+ T-cells to suppress viral replication as assessed with supernatant and intracellular p24.

Results: Compared to a DMSO control, we observed a mean 4.1-fold increase in US RNA with nanatinostat (p=0.007) and 4.8-fold increase with romidepsin (p=0.03); positive controls bryostatin and PMA/I induced 5.4- and 14.5-fold increases, respectively (p=0.03, 0.007). Neither HDACi increased MS RNA, or resulted in detectable supernatant HIV RNA, while positive controls induced both. In the biosensor assay, neither HDACi-treated CD4+ T-cell condition induced degranulation (CD107a) of either CTL clone. Bryostatin-reactivated CD4+ T-cells enabled both clones to degranulate, particularly the Nef-specific CTL (28.6FC; p=0.0003). CD4+ T-cells treated with PMA/I also induced both clones to degranulate, particularly the Gag-specific CTL (44.5FC; p=0.002). The VIAs demonstrated impairment of CD8+ T-cells by both HDACis, although to a greater degree for romidepsin versus nanatinostat (32% more; p=0.04). N-803 enhanced viral inhibition by CD8+ T-cells, with 86% reductions in the frequencies of Gag-expressing cells (p=0.03).

Conclusions: HDACi induce the accumulation of US but not MS or supernatant HIV RNA, limiting antigen presentation and recognition by CTLs, and also impair CD8+ T cell function. Our Results: highlight the importance of testing multiple features of viral reactivation to identify potent LRAs.

PP 1.13

Distinct HIV reservoir measures correlate with defective but not intact pro-viral DNA

E. Papasavvas[1], L. Azzoni[1], P. Tebas[2], K. Mounzer[3], J.R. Kostman[4], D. Richman[5], N. Chomont[6], B. Howell[7], L.J. Montaner[1]

[1] Wistar Institute, Philadelphia, USA, [2] University of Pennsylvania, Philadelphia, USA, [3] Jonathan Lax Immune Disorders Treatment Center, Philadelphia Field Initiating Group for HIV-1 Trials, Philadelphia, USA, [4] John Bell Health Center, Philadelphia Field Initiating Group for HIV-1 Trials, Philadelphia, USA, [5] VA San Diego Healthcare System and the University of California, San Diego, USA, [6] University of Montreal, Montreal, Canada, [7] Merck, Inc., West Point, USA

Background: A major priority for HIV cure strategies remains how best to measure persistence of HIV despite suppressive antiretroviral therapy (ART) in chronic HIV infection. Several assays have been developed to measure the HIV reservoir. We assessed the association between five distinct HIV measures on ART (intact and defective pro-viral DNA, integrated HIV DNA, integrated HIV Gag and Pol, and inducible RNA or p24).

Methods: Peripheral blood mononuclear cells (PBMC) from 20 HIV+ subjects chronically suppressed on ART at <50 HIV-1 copies/ml were assessed for a) intact and defective pro-viral DNA by IPDA (Accelevir), b) integrated HIV DNA by Alu-gag PCR, c) integrated HIV Gag and Pol by droplet digital PCR (ddPCR) following pulsed-field gel electrophoresis (PFGE), and d) latency re-activation in vitro measured by both cell-associated tat/rev induced limiting dilution assay (TILDA), and by HIV p24 single molecule array (Simoa). Spearman tests were used to test relationships between HIV measures.

Results: HIV DNA measures assessed by Alu-gag PCR or PFGE/ddPCR as well as in vitro latency re-activation assessed by TILDA or HIV p24 Simoa were positively associated with each other (e.g. HIV DNA measures assessed by Alu-gag PCR and in vitro latency re-activation assessed by TILDA: p=0.025, spearman's rho=0.541). On

the other hand, intact proviral DNA did not correlate with any HIV measure. However, hypermutated and/or 5′ deleted pro-viral DNA was positively associated with integrated HIV DNA assessed by Alu-gag PCR (p<0.001, spearman's rho=0.909) and total Gag by PFGE/ddPC R (p=0.008, spearman's rho=0.741), as well as with in vitro latency re-activation by HIV p24 Simoa (p=0.044, spearman's rho=0.627).

Conclusions: Alu-gag PCR or PFGE/ddPCR HIV DNA measures, as well as induced HIV p24 in HIV-1+ subjects chronically suppressed on ART best reflect hypermutated and/or deleted rather than intact pro-viral DNA.

PP 1.14

HIV integration site selection in the 3D genome: impact on viral and host gene expression

M. Benkirane

IGH. CNRS-University of Montpellier, Montpellier, France

Background: HIV integration into the host genome is essential to ensure productive infection and viral persistence in the infected cells. The aim of our study is to understand the role of genome structure and activity on HIV integration site selection and to determine the key genomic features associated with HIV integration site and their impact on viral and host gene expression.

Methods: isolated primary CD4 T cells from 4 HIV negative individuals were used to perform Chromosomal Conformation High-C (Hi-C) to identify global inter- and intra-chromosomal interactions, PCHi-C (promoter capture Hi-C) which identify the interactions between promoters and their regulatory elements within the genome, Chromatin Immunoprecipitation (ChIP) targeting repressive and active histone marks to obtain a genome wide chromatin landscape and Transient Transciptomic analyses (TT-Seq), which allow to determine transcriptionally active regions of the genome. Integrating all these genome wide data has led to a structural and functional map of resting and CD3/CD28 activated primary CD4 T cells. We used the previously described integration sites (IS) identified from cART-treated HIV infected individuals (Han et al., 2004; Ikeda et al., 2007; Maldarelli et al., 2014; Wagner et al., 2014), IS from primary CD4 T cell and cell lines infected in vitro for mapping analyses in the context of the established 3D structural and functional genomic map.

Results: Here, by integrating host epigenomic, transcriptomic data and 3D genomic structure of primary CD4 T cell, we identified key chromatin and structural features associated with HIV integration sites. Promoter capture HiC data revealed that HIV-1 target highly connected fragments within the genome and that integration sites are highly enriched in specific gene communities that we name Recurrent Integration Communities (RIC). Host epigenomic data revealed that HIV IS are highly enriched in H3K4met1 and H3K27ac epigenetic marks. Accordingly, we found that HIV-1 target preferentially regulatory elements within the genome namely enhancers and super-enhancers.

Conclusions: Our data highlight important features of HIV-1 integration sites that should be considered for the development of strategies for HIV reactivation from latency and the ability of HIV to confer an advantage to infected cells such as clonal expansion.

PP 1.15

Protein crotonylation sensitizes SMACm disruption of latent HIV by modulating the ncNF-κB signaling pathway at the step of p100 cleavage into p52

J. Guochun[1], D. Li[1], S. Falcinelli[2], L. Wong[1], C. Garrido[1], C. Galardi[3], R. Dunham[3], E. Brown[1], N. Archin[1], D. Margolis[1]

[1] UNC HIV Cure Center; Institute of Global Health and Infectious Diseases, Chapel Hill, USA, [2] UNC HIV Cure Center, Chapel Hill, USA, [3] UNC HIV Cure Center; HIV Drug Discovery, ViiV Healthcare, Chapel Hill, USA

Background: Although antiretroviral therapy (ART) effectively suppresses HIV-1 (HIV) replication, it is a life-long treatment and cannot eliminate HIV in patients. The major obstacle that impedes HIV eradication is the latent viral reservoir in resting CD4+ T cells in ART-suppressed individuals. Recently, the activation of non-canonical (nc) NF-κB pathway via targeting IAPs with SMAC mimetic (SMACm) has raised itself as an attractive mechanism to reactivate HIV from latency. Degradation of IAPs by SMACm activates NF-κB inducing kinase, leading to cleavage of p100 into p52. The latter forms a complex with RelB to stimulate transcription of its target genes after translocation into the nucleus.

Methods: Multiple HIV latency model were used, including Jurkat cell derived HIV latency models, the U1 promonocytic model of latency, primary CD4+ T cell model of HIV latency and resting CD4+ T cells isolated from HIV-positive individuals receiving ART. Protein expression was determined by Western blot. Protein network was analyzed by Mass spectrometry after anti-Pan crotonyllysine protein pulldown.

Results: We found that SMACm such as AZD5582 can disrupt latent HIV. However, its ability to reactivate latent HIV varied among different HIV latency models, with a lowest efficacy in the primary CD4+ T cell model of latency. Interestingly, SMACm disruption of latent HIV was greatly enhanced by protein crotonylation in all these in vitro and ex vivo models. This was mediated at the step of p100 cleavage into p52 in the ncNF-κB signaling pathway, leading to an enhanced RelB/p52 recruitment at the HIV promoter. Mass spectrometry analysis revealed a distinct proteome in HIV latently infected cells compared with crotonylation-reactivated cells, indicating that an ubiquitination/deubiquitination signaling axis is involved in the transcription of HIV and latency reversal.

Conclusions: We propose that a blockage of p100 cleavage prevents an efficient activation of ncNF-κB signaling pathway in the latently infected CD4+ T cells in patients. Protein crotonylation, which was recently defined by us as a new epigenetic mark for HIV transcription, potentiates the reactivation of HIV from latency by releasing the blockage of p100 cleavage, which could be exploited to develop novel strategies for HIV cure or remission in the future.

PP 1.16

Interactions with pathogenic bacteria induce HIV-1 latency in macrophages through altered transcription factor recruitment to the LTR

T. Hanley[1], V. Planelles[1], G. Viglianti[2]

[1] University of Utah Health, Salt Lake City, USA, [2] Boston University School of Medicine, Boston, USA

Background: Macrophages are infected by HIV-1 in vivo and contribute to both viral spread and pathogenesis in the host. Recent human and animal studies suggest that HIV-1-infected macrophages may serve as a reservoir that contributes to HIV-1 persistence during anti-retroviral therapy. This phenomenon may be influenced by the local tissue microenvironment, including interactions with commensal and pathogenic microbes. These effects are thought to result, in part, from engagement of Toll-like receptors (TLRs) by pathogen-associated molecular patterns (PAMPs); however, the effects of TLR signaling on virus replication in macrophages are not fully understood. We wished to determine if the sexually transmitted pathogen *Neisseria gonorrhoeae* (GC) and the gut-associated microbe *Escherichia coli* (*E. coli*), which encode TLR2 and TLR4-specific PAMPs, repress HIV-1 replication in macrophages and thereby induce a state of viral latency.

Methods: HIV-1 infected monocyte-derived macrophages (MDMs) were treated with purified TLR ligands or intact GC or *E. coli* and virus replication was measured by luciferase activity or p24 ELISA. Recruitment of transcription factors to the HIV-1 LTR was measured by chromatin immunoprecipitation assay. Production of proinflammatory cytokines and interferons were measured by ELISA. Cell surface receptor expression was measured by flow cytometry.

Results: Purified ligands that engage TLRs that signal exclusively through the adapter MyD88 activated HIV-1 expression in macrophages, whereas TLR ligands that signal through TRIF repressed HIV-1 expression. Inhibiting TLR4 signaling or blocking type 1 interferon reversed GC-mediated repression. Moreover, knock-down of TRIF fully reversed GC-mediated repression. Taken together, these

Results: demonstrate that TRIF-dependent, type 1 IFN production is responsible for the GC-mediated block to virus replication in macrophages. Finally, TLR4-mediated repression in macrophages was associated with the recruitment of interferon regulatory factor 8 (IRF8) to the interferon stimulated response element (ISRE) downstream of the 5′ LTR.

Conclusions: Our data indicate that IRF8 is responsible for repression of HIV-1 replication in macrophages in response to the TRIF-dependent signaling seen with GC and *E. coli* co-infection. These findings highlight the potential role of macrophages as reservoirs of HIV-1 and the role of the tissue microenvironment and co-infections as modulators of HIV-1 persistence.

PP 1.17

Low-level persistent/latent HIV-1 infection of macrophages corresponds to decreased NF-kB activity

T. Hanley, L. Dickey, V. Planelles

University of Utah Health, Salt Lake City, USA

Background: A significant obstacle to the eradication of HIV-1 infection is the ability of the virus to form latent infections characterized by integrated proviral DNA without viral transcription. The primary latent HIV-1 reservoir in infected hosts is memory CD4+ T cells; however, recent studies suggest that other reservoirs, including tissue macrophages, may exist. We sought to determine whether macrophages could serve as latent reservoirs for HIV-1 and, if so, which signaling pathways are involved in this process.

Methods: HIV-1 infection was monitored in *in vitro* monocyte-derived macrophages (MDMs) and *ex vivo* tissue macrophages by luciferase activity, flow cytometry, and quantitative RT-PCR. Recruitment of transcription factors to the HIV-1 LTR was measured by chromatin immunoprecipitation.

Results: The number of HIV-1-expressing MDMs peaked early after infection, and steadily decreased over time. Decreased HIV-1 expression correlated with decreased transcription and occurred in the absence of cell death, suggesting that HIV-1 enters a low-level persistent or latent state. The transition to a persistent/latent state was due in part to interferon production, as blocking type I interferon signaling partially reversed the observed decrease in HIV-1 expression. Peak virus transcription was dependent almost entirely upon NF-kB activity, as HIV-1 infection led to the nuclear translocation of the p65 subunit within four hours and virus transcription was inhibited by blocking NF-kB signaling, but not other transcription-factor signaling pathways. Both p65 and RNA polymerase II associated with the HIV-1 LTR early after infection, but these associations decreased as HIV-1 transcription decreased. In addition, a persistent low-level/latent state was induced by culture in the presence of NF-kB inhibitors. Low-level persistent/latent infection was reversed by the addition of latency-reversing agents such as TNF-a, PMA, or ingenols. Finally, tissue macrophages isolated from post-mortem splenic and liver tissue demonstrated a similar dependence upon NF-kB for HIV-1 replication.

Conclusions: Our data demonstrate that HIV-1-infected macrophages enter a state of low-level persistent/latent infection over time as NF-kB activity decreases, and suggest that macrophages contribute to the latent reservoir. These insights highlight that HIV-1-infected macrophages should be considered when developing future HIV treatment strategies.

PP 1.18

Cell proliferation contributes to the increase of genetically intact HIV over time

S. Palmer[1], B. Horsburgh[1], B. Hiener[1], K. Fisher[1], E. Lee[1], J. Milush[2], R. Hoh[2], R. Fromentin[3], N. Chomont[3], S. Deeks[2]

[1] Westmead Millennium Institute and University of Sydney, Westmead, Australia, [2] Department of Medicine, University of California San Francisco, San Francisco, USA, [3] Université de Montréal, Montreal, Canada

Background: Effective HIV eradication strategies require an understanding of the mechanisms maintaining persistent HIV during therapy. We examined the role of memory cell proliferation in maintaining genetically-intact proviruses over 4 years of effective therapy.

Methods: Naïve (N), central (CM), transitional (TM) and effector (EM) memory CD4+T-cells were sorted from the peripheral blood of two participants on long-term ART. Additional sequences from naïve, CM HLA-DR+/DR–, TM HLA-DR+/DR– and EM HLA-DR+/DR– T cells were obtained 4 years later. Full-length individual proviral sequencing was used to characterise proviruses as intact or defective. Clusters of ≥2 100% genetically identical proviral sequences - indicative of host cell proliferation – were identified.

Results: A total of 287 and 448 sequences were isolated from the first and second time-points, and 34 (12%) and 90 (20%) were considered intact. At both times the frequency of intact genomes differed between cell subsets, EM>TM>CM/N. In each subset, HLA-DR+ cells contained more intact provirus than HLA-DR- cells. The proportion of identical sequences was significantly higher in intact proviruses compared to defective at the second time-point (85% vs 41%, p=0.03), but not the first. There was a significant correlation at the second time-point between the proportion of identical sequences overall and the proportion of intact proviruses (R2=0.58-67, p=0.02-0.04). The majority (44/51, 86%) of sequences observed at both time-points (over four years) were found in cells of the same memory phenotype. A greater number of identical sequence clusters were derived from HLA-DR+ cells. However, the size of the clusters derived from cells of mixed activation status was larger, with 60% of all identical sequences derived from a cluster of both HLA-DR+ and HLA-DR- cells.

Conclusions: Genetically intact proviruses were found most frequently in the more differentiated EM cells. However, the frequency of intact proviruses was increased in each memory cell subset when the cell expressed HLA-DR, highlighting the role of cellular activation in maintaining the reservoir. Moreover, the correlation between cellular proliferation and intact provirus highlights the importance of host cell proliferation in maintaining HIV over time. These findings demonstrate the importance of limiting cellular activation, differentiation and proliferation in strategies aimed at reducing the reservoir.

PP 1.19

Intra- and inter-individual HIV diversity limits the application of the intact proviral detection assay (IPDA)

Natalie N. Kinloch[1,2]*, Yanqin Ren[3]*, Winiffer Conce Alberto[3], Winnie Dong[2], Szu Han Huang[3], Andrew Wilson[4], Talia M. Mota[3], Don Kikby[2], Perla M. Del Rio Estrada[5], Chanson J. Brumme[2,6], Guinevere Q. Lee[3], Rebecca M. Lynch[4], Zabrina L. Brumme[1,2]*, R. Brad Jones[3,4]*

*[1] Faculty of Health Sciences, Simon Fraser University, Burnaby, Canada, [2] BC Centre for Excellence in HIV/AIDS, Vancouver, Canada, [3] Division of Infectious Diseases, Weill Cornell Medical College, New York, USA, [4] School of Medicine and Health Sciences, George Washington University, Washington DC, USA, [5] Departmento de Investigacion en Enfermedades Infecciosas, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico, [6] Faculty of Medicine, University of British Columbia, Vancouver, Canada, *authors contributed equally*

Background: The Intact Proviral Detection Assay (IPDA) was developed as a quantitative, scalable assay to estimate intact HIV reservoir size and an attractive tool to evaluate the efficacy of cure interventions. IPDA's robustness to detect naturally-occurring HIV diversity however remains incompletely explored.

Methods: IPDA was used to quantify intact proviruses in CD4+ T-cells from 38 virally-suppressed participants across North America. For 28 (74%) participants, inducible reservoirs were concurrently measured by Quantitative Viral Outgrowth Assay (QVOA). Single-genome

amplification was used to recover near-full length proviruses from the reservoir and env sequences from QVOA outgrowth wells. Susceptibility of individual viruses to bNAbs was assessed by ADCC assays.

Results: No correlation was observed between intact (IPDA) and infectious (QVOA) provirus frequencies (r=-0.1, p=0.62, N=28). Notably, 7/38 (18%) participants yielded a negative IPDA result, despite recovery of replication-competent HIV in 6 of these cases. Sequencing of select 'IPDA false-negative' participants revealed that their autologous viruses harbored at least one nucleotide mismatch to the IPDA env probe. Autologous env probes rescued this signal, demonstrating that a single mismatch can yield a false negative IPDA result. Analysis of 1,045 HIV subtype B env sequences in LANL estimated that 20% of these contain at least one mismatch to the probe. An alternative RRE-targeting env primer/probe set rescued detection for all 7 'false negative IPDA' participants and detected env in 12/14 'true positive' participants. Importantly, where successful, the alternative set yielded comparable reservoir values to the original assay (p=0.2). We further identified an individual harboring heterogeneous replication-competent reservoir virus that exhibited opposing susceptibility profiles to 3BNC117 and 10-1074 by ADCC, where one of these strains was detectable by IPDA, while the other was not.

Conclusions: Given that an estimated 20% of HIV subtype B sequences could yield false-negative Results: by IPDA, caution is warranted in assay scale-up, particularly as the sole read-out in intervention trials. Alternative primers/probes can mitigate inter-individual reservoir diversity, to some extent, by reducing false-negative rates. Failure to capture intra-individual diversity however is a greater challenge, as this may lead to underestimation of reservoir size and erroneous conclusions regarding the efficacy of candidate interventions.

PP 1.20

Exploring histone loading on unintegrated HIV-1 DNA reveals the chromatin dynamics between unintegrated and integrated viral genome

M. Benkirane[1], S. Machida[1], D. Depierre[2], M. Takaku[3], O. Cuvier[2]

[1] Institut de Génétique Humaine. Université de Montpellier. Laboratoire de Virologie Moléculaire CNRS-UMR9002, Montpellier, France, [2] LBME, Centre de Biologie Intégrative, Université de Toulouse, CNRS, Toulouse, France, [3] School of Medicine and Health Sciences, University of North Dakota, North Dakota, USA

Background: The aim of our study is to understand the biology of unintegrated HIV-1 DNA and to reveal the mechanisms involved in its transcriptional silencing.

Methods: To test whether histones are loaded onto unintegrated viral DNA (vDNA), we performed chromatin immunoprecipitation experiment (ChIP) using chromatin prepared from Jurkat cells and primary CD4+ T cells infected with HIV-1. To determine nucleosome positioning on unintegrated vDNA, we performed MNase-seq analysis.

Results: Here, we show that loading of HIV-1 DNA with core histones occurs after its nuclear import and prior its integration into the host genome. Analyses of the nucleosome positioning along the unintegrated and integrated viral genome clarified major differences in nucleosome density and position. In particular, in addition to the well-known nucleosomes Nuc0, Nuc1 and Nuc2 loaded on integrated HIV-1 DNA, a nucleosome covering the DNA hypersensitive site, NucDHS, is found within unintegrated viral DNA. Additionally, Nuc0 and Nuc2 on unintegrated vDNA were found to be positioned slightly 5' of their position within integrated form. The presence of NucDHS within promoter proximal region of the LTR, blocks the accumulation of RNAPII and active histone marks H3K4me3 and H3ac at the LTR. Integration of HIV-1 DNA is accompanied by the eviction of NucDHS and sliding of Nuc0 and Nuc2, recruitment of RNAPII and gain of active histone marks within the LTR.

Conclusions: Our study shows that unintegrated HIV-1 DNA is loaded with histones in the nucleus and reveals an unexpected dynamic of the chromatin architecture between unintegrated and integrated HIV-1 DNA. Unintegrated vDNA adopts a repressive chromatin architecture, which competes with the transcription machinery and contributes to its silencing.

PP 1.21

A novel, ultra-sensitive technology for quantifying the HIV unintegrated linear DNA responsible for pre-integrative latency

J. Dutrieux[1], H. Roux[2,3], J. Migraine[4], M. Salmona[5], J. Hamroune[6], N. Arhel[7], A. Hance[8], F. Clavel[9], R. Cheynier[6]

[1] INSERM U94, Paris, France, [2] Université de Paris, Paris, France, [3] Institut Cochin, INSERM, U1016, CNRS, UMR8104, Paris, France, [4] Inserm U1259, Tours, France, [5] Université de Paris, Assistance Publique Hôpitaux de Paris, Hôpital Saint Louis, Paris, France, [6] Institut Cochin, INSERM, U1016, CNRS, UMR8104, Paris, France, [7] Institut de Recherche en Infectiologie de Montpellier, CNRS UMR 9004, Montpellier, France, [8] Inserm U941, Paris, France, [9] Assistance Publique Hôpitaux de Paris, Hôpital Saint Louis, Paris, France

Background: Viral latency is the major barrier to HIV eradication. Our study focuses on pre-integrative latency, more specifically on processed Unintegrated Linear DNA (ULD). Due to the lack of a sufficiently sensitive and specific quantification method, ULD stability has not yet been evaluated in vivo. We have successfully developed such a method, based on NGS, in order to study the dynamics of ULDs and their importance in latency.

Methods: We combined Linker Mediated PCR (LM-PCR) to a new method of ultra-deep sequencing. An adapter carrying a Unique Molecular Identifier (UMI) that acts as a molecular barcode is ligated to ULDs. LM-PCR is then performed to specifically pre-amplify ligated ULDs, followed by a second PCR round using oligonucleotides harboring Illumina sequences. After deep sequencing of the PCR products, reads carrying the same UMI are sorted – as they are considered to be products of amplification of the same ULD – and counted allowing an ultrasensitive quantification.

Results: The sensitivity of our method was first evaluated on serial dilutions of an artificial ULD. A detection threshold of 5 copies of ULD/105 cells was calculated.

We then evaluated the evolution of ULD concentration over time in MT4-R5 infected cells using either the canonic qPCR technique or our technology, and showed similar results.

Finally, in patients' samples demonstrating undetectable levels of ULDs using qPCR method, we quantified an average of 1 ULD copy/103 cells using LM-PCR + NGS method.

Conclusions: Our data shows the efficiency of a new high throughput technology to efficiently detect and accurately quantify ULDs both in in vitro infected cells and, more importantly, in patients' samples, with a very high sensitivity. Such a technology could be further used to acquire new insights on pre-integrative latency.

PP 1.22

A novel, ultra-sensitive technology for quantifying the HIV unintegrated linear DNA responsible for pre-integrative latency

J. Dutrieux[1], H. Roux[2,3], J. Migraine[4], M. Salmona[5], J. Hamroune[3], N. Arhel[6], A. Hance[1], F. Clavel[5], R. Cheynier[3]

[1] INSERM U941, Paris, France, [2] Université de Paris, Paris, France, [3] Institut Cochin, INSERM, U1016, CNRS, UMR8104, Paris, France, [4] Inserm U1259, Tours, France, [5] Université de Paris, Assistance Publique Hôpitaux de Paris, Hôpital Saint Louis, Paris, France, [6] Institut de Recherche en Infectiologie de Montpellier, CNRS UMR 9004, Montpellier, France

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Methods: We combined Linker Mediated PCR (LM-PCR) to a new method of ultra-deep sequencing. An adapter carrying a Unique Molecular Identifier (UMI) that acts as a molecular barcode is ligated to ULDs. LM-PCR is then performed to specifically pre-amplify ligated ULDs, followed by a second PCR round using oligonucleotides harboring Illumina sequences. After deep sequencing of the PCR products, reads carrying the same UMI are sorted – as they are considered to be products of amplification of the same ULD – and counted allowing an ultrasensitive quantification.

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Conclusions: Our data shows the efficiency of a new high throughput technology to efficiently detect and accurately quantify ULDs both in in vitro infected cells and, more importantly, in patients' samples, with a very high sensitivity. Such a technology could be further used to acquire new insights on pre-integrative latency.

PP 1.23

A delicate balance between the number of RBEIII and NF-κB motifs impacts latency kinetics in HIV-1

U. Ranga, D. Bhange, N. Prasad

Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India

Background: In a collaboration among five different institutes and after screening 526 primary viral strains, we report the emergence of a large number of transcription factor binding site variant HIV-1 strains over the past ten years in India. The common theme of the variation appears to modulate the transcriptional strength of the LTR by duplicating the NF-κB and RBEIII binding motifs with implications for latency. We examined the impact of RBEIII (R) and or NF-κB (N) site duplication on the latency kinetics.

Methods: We constructed panels of reporter sub-genomic or full-length viral vectors co-expressing d2EGFP and Tat under the control of canonical (RN3, one RBEIII, and three NF-κB sites) or three different variant LTRs (RN4, R2N3, and R2N4). Using flow cytometry, we evaluated the latency kinetics under diverse activation conditions using T-cell lines or long-term primary CD4 cell culture.

Results: LTRs containing RBEIII site duplication with or without NF-κB duplication (R2N3- and R2N4-LTRs) entered latency at a significantly faster rate as compared to the RN3-LTR. Importantly, latency reversal was profoundly influenced by a delicate balance between the number of RBEIII and NF-κB sites in the LTR. While the R2N4-LTR could be reversed from latency under comparable activation conditions as that of the canonical RN3-LTR, the R2N3-LTR established avid latency that could not be reversed under these conditions and needed several folds higher concentration of activators or cocktails of activators. The differential activation profiles of RN3 and R2N3 are consistent when the two viral promoters encoding two different reporter fluorescent proteins were cointegrated in a single cell. Work is in progress to evaluate the latency kinetics in a humanized mouse model, examine the latent reservoir properties in mixed infections, and characterize the transcription factor complexes.

Conclusions: LTR evolution appears to be unique for HIV-1C and a recent phenomenon. Some of the emerging strains could enjoy greater levels of replication fitness and may establish expanding epidemics in the future, which requires monitoring. A fine balance between NF-κB and RBEIII sites influences viral latency kinetics.

PP 1.24

Epigenomic characterisation of a primary cell model of HIV latency

E. Browne, B. Pace, D. Margolis, B. Strahl, R. Dronamraju, S. Jefferys, J. Parker

UNC-Chapel Hill, Chapel Hill, USA

Background: Transcriptional silencing of HIV in CD4 T cells generates a reservoir of latently infected cells that can reseed infection after interruption of therapy. As such, these cells represent the principal barrier to curing HIV infection, but little is known about the characteristics or regulation of the latent reservoir.

Methods: To further our understanding of the molecular mechanisms of latency, we employed a primary cell model of HIV latency in which infected cells adopt heterogeneous transcriptional fates with a subset of infected cells establishing viral latency. We characterized this model using assay of Transposon-Accessible Chromatin sequencing (ATACseq).

Results: We observed that loss of viral gene expression is a stable and heritable phenotype that is maintained through multiple rounds of stimulation and expansion, suggesting a role for epigenetic maintenance of latency. Using ATACseq we found that cells in which latency is established exhibit a significantly more closed chromatin conformation, both within the HIV genome and across the host cell genome, indicating that latency is correlated with a global process of epigenomic modification and heterochromatin expansion. We also observed that latency reversing agents (LRAs) induced distinct patterns of chromatin openning in both the HIV and host cell genomes. Furthermore, we observed that latently infected cells exhibited elevated levels of specific repressive histone modifications, including H3K27me3.

Conclusions: Altogether, these data demonstrate that latency establishment in primary CD4 T cells occurs preferentially in a subset of cells that exhibit expanded H3K27me3-associated heterochromatin, and that viral silencing is connected to global cellular epigenomic reprogramming. A deeper understanding of this process will likely lead to new therapeutic strategies for blocking the initiation or maintenance of latency.

PP 1.25

Proteasomal degradation of PML protein is a stress response to HIV-1 replication and reactivation

I. Shytaj[1,2], B. Lucic[1], C. Penzo[1], S. Bicciato[3], M. Forcato[3], A. Savarino[4], M. Lusic[1]

[1] Department of Infectious Diseases, Integrative Virology, Heidelberg University, Heidelberg, Germany, [2] German Center for Infection Research, Heidelberg, Germany, [3] Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy, [4] Department of Infectious and Immune-Mediated Diseases, Italian Institute of Health, Rome, Italy

Background: Promyelocytic leukemia protein (PML) is one main structural and functional component of the nucleus. PML has been described as a potential HIV-1 latency marker (Lusic et al.2013). Moreover, PML has been recently validated in vivoas a potential therapeutic target to induce a functional cure of the infection through the use of arsenic trioxide (Yang et al.2019). We investigated the impact of metabolic changes induced by HIV-1 replication and latency reactivation on PML expression.

Methods: Development of latency in vitrowas studied usingprimary CD4+ T-cells. Latency reactivation was analyzed in three different J-Lat

clones. Transcriptomic profiles were analyzed by RNA-Seq. Expression of PML was investigated by western blot and immunofluorescence coupled to 3D immuno-DNA- or RNA-FISH.

Results: RNA-Seq analysis showed that HIV-1 replication in primary CD4+ T-cells was associated with increased expression of Nrf-2 regulated antioxidant genes and enhanced iron import capacity (through transferrin receptor-1 upregulation). Upregulation of antioxidant gene expression was also observed upon HIV-1 reactivation in J-Lat cells. Both HIV-1 replication and latency reactivation were associated with PML degradation. This effect was inhibited by antioxidant supplementation (through NAC or resveratrol) or by iron chelation (through deferiprone or deferoxamine). Conversely, PML degradation could be induced by pharmacologically increasing oxidative stress or iron content. Moreover, PML degradation was dependent on proteasome function and could be inhibited by the proteasome inhibitors MG132 and bortezomib (Figure 1).

Conclusions: HIV-1 induced PML degradation is dependent on proteasome function and can be induced by increasing oxidative stress and/or intracellular iron content. Combined treatments converging on PML degradation may be able to increase the the anti-latency efficacy of arsenic trioxide.

PP 1.26

Expression of CircRNAs in HIV-1 latently infected cells from an *in vitro* model

L. Iniguez, D.C. Copertino Jr, D.F. Nixon, M. De Mulder Rougvie
Weill Cornell Medicine, New York, USA

Background: The major barrier preventing the eradication of HIV-1 is a small reservoir of latently infected CD4+ T-cells that persist after antiretroviral therapy (ART) and can spawn new waves of infection after ART cessation. These rare latently infected cells have a slow division rate, and characterization of them will guide future HIV cure strategies. Circular RNAs (circRNAs) are single stranded covalently closed RNAs which lack the characteristics of linear mRNA such as 5′cap and 3′poly-A tails. CircRNAs are formed as byproduct of post-transcriptional 'back-splicing' of coding-genes. Most transcriptomic studies in HIV-1 focus on RNA species, like mRNA or miRNA, while the expression of circRNAs remains to be elucidated. The expression patterns of circRNA have been reported to be sensitive to viral infection and cell division rates, which allow us to hypothesize that circRNAs could be found in HIV-1 latently infected cells.

Methods: We re-analyzed publicly available total RNA-seq data from an *in vitro* HIV-1 latency model. The dataset consisted of four conditions (LI: latently infected; LU: latently uninfected; LIR: latently infected reactivated; LUR: latently uninfected reactivated) from four healthy donors. Reads were mapped to identify gene expression with Hisat2 and Stringtie, then unmapped reads were used to identify circRNAs with CIRCExplorer2 and quantified with CLEAR. differentially expressed (DE) was calculated with edgeR and Results: were validated with RT-qPCR.

Results: More than 3,000 high confidence circRNAs were identified from all samples; reactivated samples showed a significant decrease in the number of circRNAs while the number of expressed genes remained similar amongst the four conditions. We focused on the comparison between LI and LU and identified 2 DE circRNAs (circPDE3B and circGTDC1) and 52 DE genes. Interestingly, the two circRNAs were significantly upregulated in LI but their corresponding gene of origin were not among the list of 52 DE expressed genes. These Results: were validated through RT-qPCR.

Conclusions: The Results: presented here greater our knowledge of the transcriptional state of persistently infected CD4+ T-cells. We have further shown that some circRNAs are differentially expressed in latent cells from an *in vitro* HIV-1 latency model. Additional studies on circRNAs in HIV-1 persistence studies are warranted.

Conflict of interest: Funding: Martin Delaney BELIEVE Collaboratory (NIH grant 1UM1AI26617); which was supported by the following NIH Co-Funding and Participating Institutes and Centers: NIAID, NCI, NICHD, NHLBI, NIDA, NIMH, NIA, FIC, and OAR. Miguel de Mulder Rougvie is funded in part by the Department of Medicine, Fund for the Future program at Weill Cornell Medicine.

PP 1.27

Cleavage and polyadenylation specific factor 6 is required for HIV latency reversal

Y. Zheng[1], A. Nau[2], V. Achuthan[3], A. Engelman[3], V. Planelles[1]
[1] Department of Pathology, School of Medicine, University of Utah, Salt Lake City, USA, [2] Department of Biochemistry, School of Medicine, University of Utah, Salt Lake City, USA, [3] Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Department of Medicine, Harvard Medical School, Boston, USA

Background: HIV can establish a status of latency in the early phase of infection both *in vitro* and *in vivo*. The mechanisms behind the HIV latency establishment and maintenance are not totally deciphered yet. To further understand the roles of host factors playing in HIV latency, we tested a preliminary panel of candidate genes which are suspected to be involved in controlling HIV-1 transcription. Cleavage and polyadenylation specific factor 6 (CPSF6) is one of our candidates, previously known to play a role in 3′ RNA cleavage and polyadenylation process and facilitate HIV integration. In this study, we further investigated CPSF6's role in HIV latency.

Methods: We have developed an HIV latency model in the lab using primary cells. Naïve CD4 cells are isolated from health donors and activated by stimulation. Pseudotyped viruses, pNL4.3-deltaEnv-nLuc-2Anef-VSVG, are used to infect activated CD4 cells. CD4+ cells, including uninfected cells and latently infected cells, are positively isolated at a later time point and subjected to CRISPR/Cas9-mediated knockout. Luciferase and P24 were analyzed after PMA stimulation to evaluate the reactivation ability of latent viruses. Flow cytometry, qPCR and western blotting were used to validate knockouts, and assess gene transcription and protein expression.

Results: Our data showed that CXCR4 (as a CRISPR knockout control), NFkb (as an HIV latency reactivation control) and CPSF6 (a candidate gene) were efficiently knocked out by CRISPR/Cas9 RNP method. Compared to CXCR4 KO group, both luciferase in the supernatant and P24+ cells after PMA stimulation of CPSF6 KO group were reduced. QPCR data revealed that the decrease by knocking out CPSF6 was not due to its effect on 3′ RNA cleavage and polyadenylation processing. A significant reduction of phosphorylated CDK9 and Pol II was observed.

Conclusions: A new role of CPSF6 was discovered in HIV infection. CPSF6 is required for HIV latency reversal.

PP 1.28

Using single-cell analysis and a primary model of HIV to study latency establishment and reactivation

L. De Armas, S. Williams, L. Pan, S. Rinaldi, S. Pallikkuth, R. Pahwa, S. Pahwa
University of Miami Miller School of Medicine, Miami, USA

Background: HIV eradication is hindered by the existence of latent HIV reservoirs in CD4 T cells (CD4). CD4 present in lymph nodes (LN) harbor high frequencies of HIV-infected cells. Here, we used a primary cell model of HIV latency and a dual reporter virus to investigate HIV latency establishment and reactivation using single cell technologies.

Methods: Purified CD4 from HIV- Tonsil donor were activated with anti-CD3/CD28 antibodies (3/28) for 3d prior to infection with HIV OGH, containing GFP under HIV-LTR promoter and mKO2 under EF1a promoter. 5 days post-infection: Live, GFP- cells were sorted and cultured for 24 hours in the presence of latency reversal agents ALT-803 (IL-15 super-agonist), Prostratin, or 3/28. Single cell sorting was performed for GFP+ and GFP- cells using BD Precise 3′ Whole transcriptome amplification (WTA) Sequences were aligned to human

and HIV genomes. Data was integrated from index sorting (flow cytometry analysis) and HIV transcript expression to define uninfected cells (GFP-mKO2-transcript-), latent infected (GFP-mKO2+transcript +/–), and productive infected (GFP+mKO2+/–transcript++). SingulaR (R package) was used to determine significant differences in gene expression.

Results: Reactivation occurred at higher frequencies in sorted GFP- cells treated with 3/28 compared to Prostratin and ALT-803 (17.9% vs 13.5% and 7.6%) as measured by flow cytometry and GFP expression. ALT-803 was not very effective in HIV reactivation compared to the negative control (7.6% vs 6.6%). In tSNE analysis, cell populations clustered by reactivation stimulus. Differential gene expression analysis showed that 801 and 125 genes were differentially expressed between reactivated (GFP+) and non-reactivated (GFP-) cells following 3/28 and Prostratin treatment, respectively. 99 of the genes overlapped between the 2 conditions and included genes related to cell metabolism and antigen processing. IL-32, a T cell produced pro-inflammatory cytokine, was expressed higher in the non-reactivated cells compared to reactivated ($p<0.001$) and control ($p<0.05$) suggesting it may be a marker of latent infection.

Conclusions: Our methods using an established model of HIV latency and single cell analysis by flow cytometry and RNA-Seq in LN-derived cells shed light on biology of latency in a crucial anatomical site for HIV persistence and are a valuable tool for investigating cure strategies.

PP 1.29

Degradation of the XPB subunit of TFIIH by spironolactone reduces HIV-1 reactivation from latency

L. Mori[1], Y.C. Ho[2], B.C. Ramirez[3], S. Valente[1]

[1] Scripps Research Institute, Department of Immunology and Microbiology, Jupiter, USA, [2] Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, USA, [3] Institut Cochin, Inserm, CNRS, Université Sorbonne Paris Cité, Paris, France

Background: HIV transcription requires the assembly of multiple cellular transcription factors and RNA polymerase II (RNAPII) at the HIV-1 5′ long terminal repeat. The TFIIH general transcription factor facilitates promoter escape by opening the DNA around the transcription start site and phosphorylating RNAPII. Spironolactone (SP), an FDA approved drug, has been shown to trigger proteasomal degradation of the XPB subunit of TFIIH. SP is known to suppress acute HIV infection in vitro. Loss of XPB does not appear to affect basal transcription, but rather selectively inhibits inducible transcription. The effects of SP treatment on HIV transcription in latently infected cells have not yet been established.

Methods: SP was evaluated as a potential 'block-and-lock' agent for a functional cure aimed at the transcriptional silencing of the viral reservoir. We investigated whether long-term SP treatment suppresses reactivation from latency upon stimulation of cells with latency reversing agents (LRAs) and upon treatment interruption. RNAPII occupancy throughout the HIV genome at basal levels and upon stimulation was also measured. Finally, we assessed the effects of SP on reactivation from latency in ex vivo resting CD4+ T cells isolated from aviremic individuals on antiretroviral therapy.

Results: SP treatment rapidly inhibits HIV-1 transcription and viral reactivation from latency in a range of latent cell lines and shRNA knockdown of XPB confirmed XPB degradation as the mechanism of action. A reduction in RNAPII throughout the HIV-1 genome was observed as a consequence of XPB degradation. Long-term treatment with SP does not result in long-lasting epigenetic suppression of the HIV promoter upon treatment interruption. Once SP is removed, viral transcription rapidly rebounds as XPB protein re-emerges. Importantly, SP inhibits HIV reactivation from latency in cell line models of latency and primary CD4+ T cells from infected individuals upon cell stimulation with LRAs.

Conclusions: We showed that targeting a host transcription factor, XPB, reduces HIV-1 transcription and reactivation from latency without obvious adverse effect on cellular transcription. XPB plays a key role in HIV transcriptional regulation and without it, initiation of transcription from latent HIV-1 genomes is impaired. These Results: strengthen the potential of HIV transcriptional inhibitors in 'block-and-lock' HIV cure approaches.

PP 1.30

Updates on two public databases for studies of HIV persistence; the Retrovirus Integration Database (RID) and HIV Proviral Sequence Database (PSD)

W. Shao[1], J. Shan[1], W.S. Hu[2], E. Halvas[3], J. Mellors[3], J. Coffin[4], M. Kearney[2]

[1] Leidos Biomedical Research, INC, Frederick, USA, [2] HIV Dynamics and Replication Program, NCI, Frederick, USA, [3] Division of Infectious Disease, University of Pittsburgh, Pittsburgh, USA, [4] Tufts University, Boston, USA

Background: Characterizing the near full-length sequences of HIV-1 proviruses and sites of host integration that persist during antiretroviral therapy (ART) is crucial to the design of potential curative interventions. To facilitate our understanding of the genetic structure and dynamics of the HIV-1 reservoir, we developed two public databases, the Retrovirus Integration Database (RID, https://rid.ncifcrf.gov) and the Proviral Sequence Database (PSD, https://psd.cancer.gov).

Methods: Both the RID and PSD were constructed with MySql (https://www.mysql.com), an open source relational database management system. Since the creation of the two databases, provirus integration sites and near full-length sequences have been added from all published studies with institutional review board approval and from donors who provided informed consent. New functions and bioinformatic tools were also added to aid in individual and meta-analyses.

Results: The RID currently includes ~6.2 million integration sites from 22 published papers, including 28,000 from HTLV-1, 82,000 from ALV, 242,000 from SIV, 1.6 million from HIV-1, and 4.3 million from MLV. Two new functions have been added to the RID, 1) ISmapper allows users to specifically map integration sites to the host genome, and 2) VIGE allows users to investigate expression levels in host genes together with integration events. The RID is currently accessed about 40 times daily. The PSD contains 1056 published near full-length HIV-1 provirus sequences obtained from donors prior to and on ART and provides detailed annotation of each sequence including inferred 'intactness'. The PSD also includes a stand-alone pipeline, ProSeq IT, that allows users to annotate and determine the inferred intactness of their own sequences without entering them into the database.

Conclusions: We have developed and updated two public databases for centralized storage and analysis of HIV-1 integration sites and near full-length proviral sequences with tools embedded for individual and meta-analyses. These databases provide the most comprehensive source of integration sites and proviral sequences available.

Conflict of interest: John Mellors is a consultant to Gilead, Merck, Accelevir Dx, and ID Connect, and share option holder of Co-Crystal Pharma, Inc.. The remaining authors have no potential conflicts.

PP 1.31

Specificity of bivalent chemical degraders targeted to the BET proteins

A-M. Turner[1], F. Potjewyd[2], A. Keller[3], L. James[2], D. Margolis[1]

[1] UNC HIV Cure Center, Departments of Medicine and Microbiology/Immunology, UNC Chapel Hill School of Medicine, University of North Carolina, Chapel Hill, USA, [2] Center for Integrative Chemical Biology and Drug Discovery, UNC Eshelman School of Pharmacy, University of North Carolina Chapel Hill, Chapel Hill, USA, [3] UNC HIV Cure Center, University of North Carolina Chapel Hill, Chapel Hill, USA

Background: PROteolysis TArgeting Chimeras (PROTACs) are bivalent molecules capable of inducing target-specific degradation. These bivalent chemical degraders contain a ligand to a protein of interest (POI) linked to a ligand for an E3 ubiquitin ligase complex. Formation of the ternary POI-PROTAC-ligase complex Results: in ubiquitination of the POI and subsequent proteasomal degradation. PROTACs represent a rapidly evolving space in chemical biology due to their catalytic nature, differences in selectivity over parent inhibitors, and target degradation versus inhibition, properties which make these molecules potentially attractive as therapeutics. Multiple potent BET (bromo- and extra-terminal domain) targeted degraders have been developed and resulted in the first compounds that can selectively target BRD4. Here we investigate the specificity of these degraders in latency models and CD4+ T-cells to further understand the role of BET proteins in HIV latency.

Methods: Five BET PROTACs, MZ1, AT1, dBET1, ARV771, and ZXH 3-26, were evaluated for their ability to degrade BRD4, BRD3, and BRD2 in Jurkats and primary CD4+ T-cells via western blot. Latency reversal was assayed via flow cytometry for GFP in Jurkat-derived latency models.

Results: All degraders tested use the pan-BET inhibitor JQ1 as the POI ligand linked to moieties that bind and recruit either the VHL or cereblon E3 ligase complexes. We demonstrate selective BRD4 degradation at certain concentrations with multiple degraders in Jurkat cells and can induce latency reversal at the same concentrations. We noted cellular toxicity at PROTAC concentrations which degraded both BRD2 and BRD4. These bivalent degraders are also functional in CD4+ T-cells at low nanomolar concentrations and interestingly, show different selectivity profiles when compared to Jurkats.

Conclusions: Here we identify 3 PROTACs, MZ1, AT1, and ZXH 3-26, which mediate BRD4 specific degradation and latency reversal in both Jurkat latency models and CD4+ T-cells. We are currently focused use of these tool molecules to further understand BET-protein involvement in HIV latency. As these molecules advance for clinical applications in oncology, the potential also exists for future use as LRAs in cure strategies.

PP 1.32

Utilising genetic barcodes to understand the role of silent integration in HIV latency

E. Larragoite[1], K.E. Kimball[1], E. Atindaana[2], A. Telesnitsky[2], V. Planelles[1]

[1] University of Utah School of Medicine, Department of Pathology, Salt Lake City, USA, [2] University of Michigan, Medical School, Microbiology and Immunology, Ann Arbor, MI, USA

Background: As HIV permanently integrates into the hosts cellular genome, we must eliminate the latent HIV-1 reservoir to cure HIV. However, the mechanisms of establishing the latent HIV-1 reservoir are poorly understood. While the latent HIV-1 reservoir has been shown to be established primarily in CD4 memory subsets, it is unclear if latency is established directly upon integration through silent integration or as memory cells transition into a resting state. In order to abolish the latent HIV-1 reservoir, we must understand the mechanisms of the establishment of HIV latency. We hypothesize that provirus integration site affects the establishment of HIV-1 latency.

Methods: In order to address our hypothesis, we are utilizing a barcoded HIV-1 library to track individual proviruses integration sites and fates. We constructed a 5′ long terminal repeat (LTR) HIV-1 barcoded library containing randomized 21-base-pair (bp) barcodes in the U5 region of the 5′LTR of NL4-3, 15 bp from the end of U5. Full length barcoded NL4-3 (BC-NL4-3) and DHIV^{NLuc2A} (BC-DHIV^{NLuc2A}) viruses were then generated and biological activity was evaluated in SupT1 and CD4$^+$ T_{CM} cells. Barcoded HIV latency studies were then conducted utilizing a primary CD4$^+$ T_{CM} latency model. Viral RNA was isolated from the supernatant overtime and cellular gDNA was isolated at the end of the experiment and prior to deep sequencing. The presence of barcodes in the supernatant were tracked overtime to determine if latency is established upon viral integration but prior to viral gene expression or at a later point as cells transition into a resting state. Trends in viral integration sites will also be evaluated.

Results: A 5′LTR HIV-1 barcoded library was constructed containing approximately 586,000 unique barcodes. The insertion of unique barcodes was confirmed upon sequencing individual colonies and viral kinetics of individual BC-NL4-3 viruses revealed the presence of replication competent viruses. The fates of individual barcoded viruses and their integration sites will be presented.

Conclusions: A 5′LTR HIV-1 barcoded library was constructed and can be used to generate replication competent viruses. Barcoded viruses were used to track fates of proviruses and investigate the role of HIV integration site in the establishment of HIV latency.

PP 1.33

Factors associated with viral control after structured treatment interruptions

N. Jilg[1], B. Etemad[2], R. Dele-Oni[2], C. Wong[2], E. Aga[3], R. Bosch[3], D. Kuritzkes[2], I. Frank[4], J. Jacobson[5], J. Li[5]

[1] Massachusetts General Hospital, Boston, USA, [2] Brigham and Women's Hospital, Boston, USA, [3] Harvard T. H. Chan School of Public Health, Boston, USA, [4] Pereleman School of Medicine, Philadelphia, USA, [5] Case Western Reserve University, Cleveland, USA

Background: In the CHAMP study, HIV post-treatment controllers (PTCs) were found to be enriched among individuals randomized to receive intermittent structured treatment interruptions (STIs) in the A5068 trial. We aimed to identify virologic determinants of post-treatment control in the participants of A5068 who underwent STIs.

Methods: A5068 participants in the STI arms underwent two short (~4 weeks) TIs (STI 1 and 2) and a subsequent extended analytical TI (ATI). Both STI 1 and 2 were followed by 16 weeks of ART. We compared plasma viral load (pVL) dynamics after each STI between PTCs and post-treatment non-controllers (NCs). Single-genome sequencing (SGS) of pol from plasma HIV RNA was performed for 6 PTCs and 7 NCs. Confirmatory long-range SGS of the pol-env region was performed for a subset of time points. Viral diversity was calculated by the average pairwise distance (APD) at one time point and viral divergence was calculated by APD between sequences of different time points.

Results: For both the first and second STI, PTCs (n=6) had significantly lower peak pVLs compared to NCs (n=27; median pVL [Q1, Q3] for PTCs vs. NCs at the first STI: 1,270 [536, 5,593] vs. 37,506 [1,643, 66,579] HIV-1 RNA copies/mL, p=0.001; and second STI: 199 [<50, 424] vs. 14,562 [7,870, 33,031] HIV-1 RNA copies/mL, p=0.001). A retrospectively-derived algorithm that used a combination of peak pVL<10,000 HIV-1 RNA copies/mL during STI 1 and peak pVL<1,000 HIV-1 RNA copies/mL during STI 2 accurately predicted that all 6 PTCs would achieve HIV control and that 26/27 NCs would not. In addition, we have generated >500 plasma HIV single-genome sequences for the PTCs and NCs during the TIs. Higher plasma HIV diversity during STI 1 correlated with higher diversity in ATI (Spearman r=0.67, p=0.02). Increasing viral divergence from STI 1 to ATI was associated with a higher peak pVL at ATI (Spearman r=0.69, p=0.02).

Conclusions: In participants undergoing STIs, lower peak pVLs during the first two short TIs may predict post-treatment control. Emergence of divergent viral populations during the third TI may compromise the ability to achieve viral control.

Conflict of interest: NJ: spouse – salary from Celgene

PP 1.34

Visualisation of HIV reactivation from latency in primary resting memory T-cells

F. Kizito, J. Karn, U. Mbonye, C. Dobrowolski, S. Valadkhan

Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, USA

Background: We have developed a primary cell model for HIV latency, called QUECEL (Quiescent Effector Cell Latency) which generates a large and homogenous population of latently infected $CD4^+$ memory cells. By purifying HIV infected cells and inducing cell quiescence with a defined cocktail of cytokines, we have eliminated the largest problems with previous primary cell models of HIV latency, namely: variable infection levels, ill-defined polarization states, and inefficient shut down of cellular transcription.

Methods: High-resolution Deltavision microscopy was used to track the induction of P-TEFb, 7SK snRNA and their assembly with Tat during time courses following T-cell stimulation leading to the reactivation of latent HIV. Stelleris probes were developed to the 3' UTR and RRE to differentiate between HIV transcripts.

Results: The quiescent cells display a distinct cellular transcription profile, and in addition to shutting down much of their metabolism, the sequestration of the transcription initiation factors NFκB and NFAT and the blocking the assembly of the host elongation factor P-TEFb prevents HIV transcription. Assembly of functional P-TEFb is initiated by exchange of CDK9 from an Hsp90/Cdc37 cytoplasmic complex to the 7SK RNP complex which requires pThr186 phosphorylation. Subsequently, there is an exchange of P-TEFb between the 7SK RNP complex and Tat that is regulated by CDK7 phosphorylation of Ser175. In situ hybridization of HIV transcripts show the initiation of HIV transcription phase separated nuclear compartment enriched in the 7SK snRNP complex. Surprisingly, RRE-containing transcripts transient though the nucleolus, a phenomenon seen decades ago in transformed cells but largely regarded as an over-expression artifact.

Conclusions: To reverse HIV latency both activation of P-TEFb and induction of HIV transcription initiation is required. The ordered assembly of the critical transcriptional elongation factor P-TEFb leads to creation of nuclear 'transcription factories' that associate with the HIV provirus

PP 1.35

An unbiased platform to identify regulators of HIV latency in infected primary human CD4 T Cells

U. Rathore[1,2], J. Hiatt[1], D.A. Cavero[1], A. Marson[1]

[1] *Department of Microbiology and Immunology, University of California San Francisco, San Francisco USA,* [2] *Diabetes Center, University of California San Francisco, San Francisco, USA*

Background: Despite nearly 40 years of extensive research, a cure for HIV remains elusive. The clearest barrier to an HIV cure is the 'latent reservoir,' a pool of host cells carrying functional HIV provirus within their genomes but not expressing HIV proteins, rendering these cells invisible to current therapeutics. Our limited understanding of how HIV production is regulated in these cells, in turn, limits the potential therapeutic targets we can manipulate and demonstrates a need for unbiased screening approaches that closely mimic in vivo biology. Unfortunately, genome-scale unbiased functional genetic studies in primary human immune cells have been largely impossible until recently.

Methods: To identify the role of host genes in the regulation of HIV latency, we have developed a new method for pooled screening in latently-infected primary human CD4+ T cells. We have designed a replication-competent HIV-1 virus that harbors a CRISPR guide RNA expression cassette. Infection of activated or resting primary cells with this virus followed by electroporation with purified Cas9 protein allows for pooled gene knockout specifically in HIV-infected cells.

Results: This procedure is compatible with several well-established primary models of latency. Edited primary CD4+ T cells are assessed for reactivation in the presence or absence of diverse potential latency-reversing stimuli. Since the gRNA cassette is embedded within the HIV genome, it functions as a genetic barcode, eschewing the need for the costly, laborious and time-consuming sorting of cells that typically limits the throughput of pooled screens. Instead, bulk deep sequencing of proviral DNA and full-length HIV RNA is sufficient to assess the effect and magnitude of latency reactivation after a given genetic perturbation. We are now conducting screens in multiple models of latency.

Conclusions: This approach will greatly expand the scope of our understanding of the pathways that control HIV latency in human T cells and can uncover novel therapeutic targets for both reactivation- and repression-mediated cure strategies.

PP 1.36

HIV-1 genomes cluster in nuclear niches of human macrophages

F. Di Nunzio

Institut Pasteur, Paris, France

Background: Productive infection by the HIV-1, the causative agent of AIDS, requires reverse transcription (RT) of the viral RNA (vRNA) genome into double-stranded viral DNA (vDNA), followed by integration of the vDNA into host cell chromosomes. These two key processes in the viral replication cycle are considered to be separated not only in time but also in cellular space, as RT is thought to be completed in the cytoplasm and a prerequisite to nuclear import and chromosomal integration. However, previous studies of the cellular route of HIV-1 have mainly focused on dividing cells. Yet, it is not known whether the replication cycle proceeds differently in other cell types, such as non-dividing cells. Indeed, different cell types can exhibit widely divergent responses to viral attacks. Macrophages are terminally differentiated, non-dividing cells derived from blood monocytes, which play a critical role in the innate and adaptive immune response. Along with activated CD4+ T cells, macrophages are also natural target cells for HIV and accumulating evidence points to a critical role of these cells in viral persistence, which remains a major roadblock to a cure for AIDS. Our aim was to investigate the exact location and timing of early steps of viral life cycle in macrophages.

Methods: We used fluorescence microscopy techniques to visualize and quantify vDNA and vRNA in infected human macrophages.

Results: We show that infected macrophages display large nuclear foci of viral DNA and viral RNA, in which multiple genomes cluster together. These clusters form in the absence of chromosomal integration, displace the paraspeckle protein CPSF6 and localize to nuclear speckles. Surprisingly, viral RNA foci consist mostly of genomic, incoming RNA, and are niches of viral life cycle functions. These findings change our view of the spatiotemporal events of the early HIV-1 replication cycle in macrophages and may contribute to understand the mechanism underlying the persistence of HIV-1.

Conclusions: Our study sheds new light on the early replication cycle of HIV-1 in macrophages. Our discovery of clusters of viral RNA and DNA opens new perspectives for understanding and combating HIV-1 persistence and replication in natural target cells.

Conflict of interests: This work was supported by Fondation pour la Recherche Medicale, Sidaction (VIH20170718001, ANRS (grants N. ECTZ4469-ECTZ74440) and Pasteur Institute

Session 2: *In vitro* and animal model studies of HIV persistence

OP 2.2

Barcoded viruses facilitate tracking changes to the composition of the rebound-competent reservoir

B. Keele, T. Immonen, C. Fennessey, J. Lifson

AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Frederick, USA

Background: To increase analytical resolution of the viral dynamics underlying establishment and persistence of the rebound competent reservoir (RCR), we used a barcoded virus (SIVmac239M) to assess the reactivation of individual viral lineages during successive analytic treatment interruptions (ATIs).

Methods: Four rhesus macaques infected intravenously with 10,000IU of SIVmac239M received combination antiretroviral therapy (cART) initiated at 10 days post infection (dpi). Three ATIs of 21 days or less were initiated at 313, 444 and 682 dpi in each animal, with the second and third ATI preceded by antibody-mediated, CD8+ lymphocyte depletion 3 days prior to cessation of treatment. Plasma viremia was suppressed to below 15 copies/mL for at least 3 months between ATIs, and cART was resumed after peak viremia was reached during viral recrudescence. Next generation sequencing was used to assess the distribution of barcodes in plasma during primary infection and during each viral rebound.

Results: Infection with many distinct barcodes was established in each animal, with a mean of 599 (557 to 650) barcodes observed pre-cART (10 dpi). The magnitude and diversity of viral recrudescence was the highest during the second ATI in each animal, with a mean of 109 (89 to 133) detectable lineages, vs 22 (8 to 34) and 27 (8 to 37) barcodes during the first and third ATIs, respectively. The probability of detection of individual barcodes during each ATI increased proportionately to their frequencies at 10 dpi, demonstrating the establishment of a large RCR representative of pre-cART viral lineages. However, in three out of four animals, the proportion of barcodes shared between consecutive ATIs was significantly higher than expected based on their relative frequency before cART, indicating enrichment of reactivated barcodes in the RCR following ATI.

Conclusions: The barcoded virus allowed assessment of the contribution of individual viral lineages to the RCR, and changes in composition of the reservoir over time. Evaluating barcode dynamics by sequencing viral DNA during suppressive cART may help define the roles of clonal expansion and reservoir re-seeding during ATIs in the observed enrichment of certain barcodes.

OP 2.3

Delay in viral rebound with TLR7 agonist, N6-LS and PGT121 in SHIV-infected macaques

D. Hsu[1], D. Silsorn[2], R. Imerbsin[2], A. Pegu[3], J. Mascola[3], R. Geleziunas[4], R. Koup[3], D. Barouch[5], N. Michael[6], S. Vasan[6]

[1] *Mlitary HIV Researrch Program (MHRP), Bangkok, Thailand,* [2] *Armed Forces Research Institute of Medical Search, Bangkok, Thailand,* [3] *National Intsitute of Health, Bethesda, USA,* [4] *Gilead Sciences, Foster City, USA,* [5] *BIDMC, Boston, USA,* [6] *MHRP, Silver Spring, USA*

Background: Toll-like receptor (TLR)-7 agonist and PGT121 administration have previously delayed viral rebound and induced SHIV remission after antiretroviral therapy (ART) interruption in macaques that started ART 7 days post SHIV-SF162P3 infection. We evaluated the impact of TLR-7 agonist and dual broadly neutralizing antibodies (bnAb) on viral rebound in SHIV-infected macaques.

Methods: Male rhesus macaques (n=16), pre-screened to exclude protective MHC alleles, were inoculated at wk0 with SHIV-1157ipd3N4 intrarectally. ART (tenofovir, emtricitabine and dolutegravir) was initiated on Day14. Active arm (n=8) animals received oral GS-986, every 2 weeks from wk14-32 and intravenous N6-LS and PGT121 every 2 weeks from wk24-32 unless anti-drug antibodies (ADA) developed. ART was ceased when plasma levels of N6-LS and PGT121 were <0.25mg/mL. Control animals (n=8) received intravenous saline. Plasma SHIV RNA was assessed by qPCR (limit of detection 10 copies/mL) and soluble markers of immune activation by multiplex assay using Luminex.

Results: All animals were SHIV-infected with median SHIV RNA of 5.7 (range 4.1-6.8) log10copies/mL on day14. After ART initiation on day14, SHIV RNA became undetectable in all animals by wk8 and remained undetectable until ART interruption. Due to varying ADA, animals received 7-10 doses of GS-986, 2-5 doses of PGT121 and 2-5 doses of N6-LS. At 24hrs post GS-986 dosing, plasma levels of IFNα, IL-1RA, IL-2, IL-6, IL-10, IL-15, MCP-1, MIP-1b, TNF, GM-CSF, IL-13 and MIP-1a increased significantly and viral blips were not detected. In the active arm, %Ki-67+ NK cells also increased at wk24 when compared to wk14 (p=0.031). Total HIV DNA levels in PBMC prior to ART interruption were not significantly different between arms. Median time to viral rebound was 6 weeks in active arm and 3 weeks in control arm (p=0.024, Figure 1). There was no significant difference in post rebound peak or set-point viremia between groups.

Conclusions: Administration of GS-986 and dual bNAbs was associated with a modest delay in viral rebound. The effect of timing of ART initiation on seeding of the viral reservoir likely influenced the ability to achieve remission. Evaluating this strategy in humans is warranted.

OP 2.4

Chimeric antigen receptor T Cells and stem cells control and protect against SHIV replication in non-human primates

C. Peterson[1], B. Rust[1], A. Zhen[2], K. Brandenstein[1], N. Poole[1], C. Maldini[3], G. Ellis[3], S. Kitchen[2], J. Riley[3], H.P. Kiem[1]

[1] *Fred Hutchinson Cancer Research Center, Seattle, USA,* [2] *University of California, Los Angeles, USA,* [3] *University of Pennsylvania, Philadelphia, USA*

Background: To date, the only clinical cases of sustained antiretroviral therapy (ART)-free HIV-1 remission have followed hematopoietic stem and progenitor cell (HSPC) transplantation. In contrast to HSPC approaches, adoptively transferred chimeric antigen receptor (CAR) T-cells have enabled long-term remission in cancer, and are likely applicable to HIV-1. Although T-cell immunotherapies currently display a superior safety profile, the duration of antiviral function *in vivo* may be limited. We have previously described an HSPC-based CAR approach that supports lifelong persistence of HSPC-derived CAR T-cells but is limited by the cytotoxic conditioning regimen that is required for HSPC engraftment. To compare and iteratively optimize HSPC- and T-cell-based immunotherapies for HIV cure studies, we employed our well-established non-human primate (NHP) model of HIV gene therapy.

Methods: Second generation CD4-based CAR molecules (CD4CAR) were independently developed for primary NHP HSPCs and T-cells, and combined with CCR5 editing or virus fusion inhibitors to protect against infection with simian/human immunodeficiency virus (SHIV). Where applicable, SHIV-infected animals were suppressed by ART for at least 1 year prior to cell therapy. CD4CAR T-cell products were infused without a conditioning regimen. CD4CAR HSPC studies were designed to identify the reduced-intensity conditioning regimen with the highest ratio of antiviral impact to cytotoxicity.

Results: Infusion of cell-based antigen significantly increased the percentage of peripheral blood CAR^+ T-cells, which persisted *in vivo* and significantly delayed SHIV rebound following ART withdrawal. Plasma viremia in one animal remains at the limit of detection 60 days post-ART treatment interruption (ATI). CD4CAR HSPC products engrafted following busulfan conditioning with minimal toxicity, and busulfan/CD4CAR-HSPC animals resisted repeated low-dose intrarectal SHIV challenges.

Conclusions: Our augmented NHP cell manufacturing platforms, antigen boosting strategies, and reduced-intensity conditioning

regimens have contributed to extremely promising Results: *in vivo*. To our knowledge, these studies are the first to i) demonstrate expansion of virus-specific, autologous CAR T-cells in infected, suppressed hosts, ii) delay/control post-ATI viral rebound following CAR T-cell therapy, and iii) enable resistance to repeated mucosal SHIV challenges following CAR-HSPC therapy. These preclinical findings are already under investigation in analogous clinical trials utilizing CAR T-cells (University of Pennsylvania NCT03617198) and busulfan/gene-edited HSPCs (City of Hope NCT02500849).

OP 2.5

The latency reversal activity of the SMAC mimetic AZD5582 in ART-suppressed SIV-infected rhesus macaques is potentiated by CD8a cell depletion

M. Mavigner[1], A. Brooks[1], C. Mattingly[1], T. Vanderford[1], B. Keele[2], J. Lifson[2], R. Dunham[3], D. Margolis[3], G. Silvestri[1], A. Chahroudi[1]

[1] Emory University, Atlanta, USA, [2] Frederick National Laboratory for Cancer Research, Frederick, USA, [3] UNC Chapel Hill, Chapel Hill, USA

Background: A leading approach to reduce viral reservoirs, referred to as 'kick and kill', aims to induce HIV expression from latently-infected CD4+ T-cells and subsequently clear the cells that have reactivated the virus. Latency reversal agents (LRAs) tested to date have been only modestly effective. Targeting the non-canonical NF-kB pathway (ncNF-kB) with small molecule mimetics of the second mitochondrial activator of caspases (SMACm) is a promising LRA approach in vitro. Furthermore, as CD8+ cells contribute to the maintenance of viral suppression during ART, experimental depletion of CD8+ cells may act synergistically with LRAs in inducing virus production.

Methods: We characterized the in vivo LRA activity of the SMACm AZD5582 in ART-suppressed SIV-infected rhesus macaques (RMs) in the presence or absence of CD8+ cells. Eighteen SIVmac239-infected RMs on ART for 55-67 weeks were administered weekly i.v. doses of AZD5582 at 100 mg/kg. Six RMs also received a single dose of the CD8α-depleting antibody MT-807R1 at 50 mg/kg 24h prior to AZD5582 treatment. On-ART plasma viral loads were monitored to assess for latency reversal and single genome sequence analysis of the env gene of SIVmac239 was performed in the plasma.

Results: On-ART viremia > 60 copies/ml was observed in 5/12 (42%) RMs treated with AZD5582 only. Ultrasensitive plasma viral load assessment (>3 copies SIV RNA per ml of plasma) showed increased plasma viremia in 3 additional RMs treated with AZD5582. Phylogenetic analyses of plasma viruses showed several patterns of virus sequences, suggesting that AZD5582 induced virus reactivation from a diverse population of cells, some of which were likely clonally expanded. When experimental depletion of CD8a+ cells was combined with AZD5582 treatment, 6/6 (100%) RMs demonstrated on-ART viremia > 60 copies/ml. These episodes of viremia above 60 copies/ml were seen in 25% versus 16.7% of viral load measurements for the CD8-depleted+AZD5582 group vs. the AZD5582 only group, respectively.

Conclusions: These studies show that activation of ncNF-kB signaling pathway via AZD5582 Results: in SIV-RNA expression in the blood of SIV-infected, ART-suppressed RMs that can be potentiated by CD8+ cell depletion.

OP 2.6

Utilising correlative PET/CT and multiscale imaging to define the dynamics of SIV-infected cells from early ART initiation to the rebound after analytic treatment interruption

T. Hope[1], M.S. Arif[1], Y. Thomas[1], I. Clerc[1], A. Carias[1], E. Allen[1], M. Mcraven[1], M. Ramirez[2], P. Santangelo[3], F. Villinger[2]

[1] Northwestern, Chicago, USA, [2] New Iberia Research Center, New Iberia, USA, [3] Georgia Tech, Atlanta, USA

Background: THe oragins of Human immunodeficiency virus (HIV) persistence during ART is controversial with a major focus on long-lived, latently infected memory T-cells while other models suggest a major role for infected myeloid cells in viral persistence. Here we explore the dynamics of viral infected cells with a PET/CT 64Cu-FAB2 probe (7D3) which allows iterative imaging of the same animal to quantitatively localize SIV envelope after initiation and cessation of ART.

Methods: RMs were challenged both vaginally and rectally with a single high-dose of SIVmac239ff (contains firefly luciferase reporter) and suppressive ART was initiated 4 days post-challenge. Following at least 6 months of ART, treatment was discontinued and RMs were necropsied at days 4, 5, 7 and 10 post-ART cessation. During and after ART cessation, PET/CT scans revealed the sites of SIV gene expression. This was validated by using the PET/CT signal to guide the isolation of small pieces of tissues at the time of necropsy which contained SIVmac239 infected cells by PCR and immunofluorescense staining.

Results: SIV infections sites were efficiently detected by PET/CT as early as four days post-ART cessation in multiple tissues particularly genital tract, small bowel and colon, slpeen, lymphnodes, and unexpectedly the heart. Interestingly, rebound was primarily observed in the same anatomical sites where signal was localized after initiation of ART. Initial phenotyping studies of rebound site have surprisingly only revealed SIV Envelope and Gag positive myeloid cells. No infected CD4+ T cells have been detected although they are abundant in the area of rebound often contacting the SIV expressing myeloid cells.

Conclusions: These observations reveal that there may be a unique host environment during the early period of rebound after ATI that restricts replication and spread to tissue resident myeloid cells. Detailed characterization of these myeloid cells will provide importantly new insights into the viral rebound in the SIV model. This restriction of the early rebound to tissue resident myeloid cells may represent a bottle neck, and potential vulnerability, required for virus replication independent of the source of the reservoir. We demonstrate correlative PET/CT is a powerful tool to define the anatomy and physiology of HIV persistence.

PP 2.1

Elimination of HIV-1/SHIV infected cells by combinations of bispecific HIV x CD3 DART® molecules

M. Tuyishime[1], J. Pickeral[1], N. Jeffrey[2], C. Ann[3], S. Guido[4], M. David[5], F. Guido[1]

[1] Department of Surgery, Duke University Medical Center, Durham, USA, [2] MacroGenics, Rockville, USA, [3] Yerkes National Primate Research Center, Emory University, Atlanta, USA, [4] Department of Pediatrics, Emory University, Atlanta, USA, [5] UNC HIV Cure Center, Departments of Medicine, Microbiology and Immunology, and Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, USA

Background: DART molecules that coengage HIV-1 envelope (Env) on infected cells and CD3 on cytolytic T cells are a strategy to eliminate infected, Env-expressing cells. Using human cells infected *in vitro* and a novel *ex vivo* system with cells from infected rhesus macaques (RMs), we tested for DART combinations best suited for administration to infected RMs *in vivo*.

Methods: DART molecules targeting HIV-1 Env at C1/C2 (A32), V1/V2 (PG9), V2 apex (PGT145), V3 glycans (PGT121) and gp41 (7B2) were evaluated for binding and redirected killing activity. *In vitro* killing was assessed with 7 transmitted/founder (T/F) HIV-1 infectious molecular clones (IMCs) containing a *luciferase* gene: CH040, CH505, DU151, SUMA, DU422, TV-1, 1086.c. Primary human CD4 cells infected *in vitro* (targets) and autologous CD8 cells (effectors) were incubated with DARTs and killing measured by decrease in luciferase. *Ex vivo* killing was conducted with primary CD4 cells from 5 SHIV.CH505.375H-infected RMs obtained at peak

of viremia (targets) and autologous CD8 cells obtained after RMs were put on ART and viremia was undetectable (effectors). Following activation *in vitro*, CD4 cells were incubated with effectors and DART molecules, then cultured with A66 cells to expand replicating virus, measured by p27 ELISA.

Results: *In vitro* killing: A32+7B2 DART combination mediated the best activity against cells infected by CH040 IMC compared to any individual DART. A32+PG9 or A32+7B2 DART combinations increased the killing of cells infected by SUMA, DU151, DU422, TV-1 or 1086.c IMCs compared to A32 DART alone. PGT145 DART showed the most binding to and mediated the highest killing of CH505.IMC-infected cells. *Ex vivo* killing: At day 4, PGT145 DART was the most active, but at day 11, A32+7B2 DART combination achieved activity similar to PGT145 DART, which was greater than A32 or 7B2 DART alone. The triple DART combination (A32+7B2+PGT145) exhibited the greatest activity.

Conclusions: We developed a novel *ex vivo* primary RM autologous system to determine the sensitivity of cells from SHIV.CH505.375H-infected RMs to combinations of DART molecules. We will analyse whether *in vivo* testing of the triple DART combination (A32+7B2+PGT145), with 2 non-neutralizing and 1 neutralizing anti-Env specificities, will recapitulate our *in vitro* results.

PP 2.2

Subtype and tropism can influence the establishment and inducibility of latent HIV-1 in primary CD4 T cells

I. Sarabia[1], S.H. Huang[2], A.R. Ward[2], R.B. Jones[2], A. Bosque[1]

[1] *George Washington University, Washington, USA,* [2] *Weill Cornell Medicine, New York, USA*

Background: Within HIV-1, there are over 10 viral subtypes. Studies evaluating factors that influence the establishment and inducibility of the latent reservoir have been mostly focused on subtype B viruses, though only 11% of worldwide infections are subtype B. As such, it is not completely understood whether subtype may have an impact on proviral latency or latency reversal. Furthermore, it has not been fully evaluated whether virus tropism may influence the establishment of an inducible latent reservoir in memory CD4 T cells. In this work, we have evaluated subtype and tropism as variables in latency establishment and reversal using a well-characterized in vitro primary cell model, the TCM model.

Methods: We used replication competent R5-tropic viruses AD8 (a subtype B virus) and MJ4 (a subtype C virus) to generate latently infected cells using the TCM model and compared to the X4-tropic virus NL43 (a subtype B virus). We characterized the establishment of latency and the inducibility of latent proviruses for a panel of well characterized Latency-Reversing Agents (LRAs). We measured the establishment of latency using digital droplet PCR for total gag and the Intact Proviral DNA Assay (IPDA) to estimate the percentage of intact proviruses. Inducibility of latent proviruses was measured using flow cytometry.

Results: We found that NL43, AD8 and MJ4 established latency in this primary cell model of latency albeit to varying degrees. Compared to NL43, AD8 generated a smaller proportion of intact proviruses. In spite of that, we observed that AD8 was more inducible than NL4-3 to various LRAs, while MJ4 exhibited an intermediate phenotype. Finally, our Results: indicate that less than 10% of intact proviruses can be reactivated even in the context of maximal stimulation.

Conclusions: We have shown that R5-tropic viruses can be used in this primary cell model of latency and can be extended to include other subtypes besides subtype B. Our Results: suggest that tropism and subtype may influence establishment of latency and its reversal. Additionally, the panel of LRAs tested were largely ineffective at inducing intact latent proviruses. The mechanisms underlying the differences in low inducibility observed are being investigated further.

PP 2.3

Biomarkers to predict reactivation from latency in SIV infection

M. Pinkevych[1], M. Davenport[1], C. Fennessey[2], C. Trubey[2], K. Brandon[2]

[1] *UNSW Sydney, Sydney, Australia,* [2] *Frederick National Laboratory for Cancer Research, Frederick, USA*

Background: A major question in HIV 'cure' research is how to measure the effects of anti-latency treatments. ATI studies have been carried out aimed to detect delays in viral rebound, and a variety of assays have been developed to measure the HIV 'reservoir' in peripheral blood. However, there is as yet no clear biomarker that can predict time-to-rebound after ATI.

Methods: We have developed a barcoded SIVmac239M that allows for the infection of macaques and treatment at different times post-infection (on days 4, 10, and 27) to create different sized SIV 'reservoirs'. Using high throughput sequencing of virus we can identify the progeny of individual 'reactivation founder' SIV clonotypes, and use mathematical modelling to estimate the frequency of reactivation from latency. We use this model to investigate the establishment of latency in early infection, and how different potential measures of reservoir size correlate with the frequency of SIV rebound after treatment interruption.

Results: We find that the frequency of SIV reactivation from latency after ATI varies from 0.4 to 3.1 reactivation events per day in animals treated on different days. The frequency of reactivation is established early, since animals treated on day 4 have only half the frequency of reactivation of animals treated on day 27 post-infection (0.61 / day vs. 1.33 /day), despite the day 27 treated animals having >100 fold more SIV DNA per million PBMC. The major difference between DNA levels and reactivation frequency is not explained by differences in SIV RNA, immune activation, CD8+ T cell responses, or replication competence.

Conclusions: This study confirms previous observations that latency is established early post-infection (day 4), but show that prolonging the duration of infection to day 27 only increases the frequency of reactivation by 2-fold. The major increase in SIV DNA in PBMC between day 4 and day 27 with only a small increase in reactivation frequency suggests that DNA measured in peripheral blood is not a direct measure of the reservoir responsible for post-ATI reactivation. This is consistent with published studies in HIV, where treatment in Fiebig 1 was associated with only small delays to viral rebound post-ATI.

PP 2.4

Effect of natural killer cells on viral rebound in HIV-1-infected humanized mice

J. Kim, C. Carmona, K. Farrell, T.H. Zhang, H. Chen, M. Dimpasoc, M. Soliman, M. Marsden, R. Sun, J. Zack

University of California Los Angeles, Los Angeles, USA

Background: Although antiretroviral drugs (ARV) are extremely effective at suppressing HIV replication, they are not curative. If ARV are discontinued for any reason in HIV-infected individuals, rebounding viremia and renewed disease progression ensue because of reservoirs of infected cells that persist despite suppressive ARV. Natural killer cells (NK cells) are innate immune effectors cells that play a critical role in the eradication of viral infections and development of adaptive immunity. It is clear that NK cells exert immune pressure on HIV and contribute to protective vaccine responses and important immunoregulation. We hypothesized that NK cells influence viral rebound and the latent reservoir.

Methods: First, to test whether human peripheral NK cells could inhibit acute HIV infection in vivo. We engrafted immunodeficient NOD-scid IL2rgnull (NSG) mice with human NK-depleted PBMC, then infected them with the HIV-1 isolate NL4-3 in the presence or absence of NK cell injections. We also tested whether NK cells can decrease viral rebound in an HIV latency model in vivo. Humanized BLT (bone marrow, liver, thymus) mice were infected with NL4-3,

then suppressed on an oral ARV regimen to generate a latent reservoir in vivo. ARV was interrupted in the presence or absence of NK cells and viral rebound was monitored.

Results: We found that NSG mice receiving NK cells had a significant delay to acute infection and viremia compared to mice that did not receive NK cells. These Results: demonstrate that even without the administration of exogenous human cytokines, peripheral NK cells are able to engraft sufficiently, albeit transiently, to delay acute HIV infection in vivo. In addition, we found that NK cell injections significantly delayed viral rebound following ART interruption in an NSG-BLT model of HIV latency in vivo.

Conclusions: We conclude that human peripheral NK cells inhibit acute HIV infection and delay viral rebound in humanized mouse models. These Results: underline the promising potential of NK cell-based therapies for new and adjunctive therapeutic approaches to combat HIV disease.

PP 2.5

Cell-associated provirus size decreases in the xenografted CD4 T cells from chronically infected natural host in a novel simianized mice model

Z. Yuan[1], S. Lai[2], K. Portion[2], R. Fast[3], I. Ourmanov[2], A. Buckler-White[2], J. Lifson[3], J. Brenchley[3], L. Montaner[1], V. Hirsch[2]

[1] Wistar Institute, Philadelphia, USA, [2] NIH, Bethesda, USA, [3] NCI, Frederick, USA

Background: Human immunodeficiency virus arose from cross-species transmission from African primates. Natural hosts of simian immunodeficiency virus usually undergo a non-pathogenic outcome and stable CD4+ T-cell counts. To gain insights into the mechanism of controlling the pathogenesis of natural hosts, we evaluated viremia, cell-associated provirus size and CD4 T-cell change following adoptive transfer of PBMC or flow-sorted lymphocyte populations from infected African green monkey (AGM) or rhesus macaques (RM).

Methods: Following the adoptive transfer of PBMC or flow-sorted NHP lymphocyte populations from infected NHPs into NSG mice, we established a simianized mouse model to track immune and viral changes within different sorted cell populations. Cell-associated provirus size was analyzed in simianized mice with AGM infected with SIVagm as compared to a RM pathogenic model infected with SIVsm. Lymphocyte change over time (frequency of CD4, apoptosis) was measured. GVHD was also analyzed against the comparable transfer of human lymphocytes into NSG mice.

Results: Simianized mice have superior reconstitution than humanized mice with sorted lymphocyte populations, >20 weeks without GVHD. Almost all AGM CD4+T cells will transform to CD8aa T cells irreversibly in AGM derived simianized mice. CD4+T cells from SIV positive AGM can deplete but slower than the CD4+T cells from SIV positive RM in simianized mice. Plasma viral load kinetics are consistent with CD4+T cells kinetics rather than with CD8aa T cells kinetics. While CD4+ T-cells transformed into the CD8aa cells, the cell-associated proviral size dramatically decreased in AGM derived simianized mice.

Conclusions: Our work establishes a novel simianized mice model for tracking immune and viral changes in SIV infection using cells from NHPs. Our finding on the change in proviral size in AGM and retention of CD8aa cells, as compared to RM, highlights a mechanism for eradicating provirus and pathogenesis in natural hosts.

PP 2.6

μ-Lat: a high-throughput humanised mouse model of latent HIV infection

H. Sperber, P.P. Togarrati, K. Raymond, M.S. Bouzidi, R. Gilfanova, M.O. Muench, S.K. Pillai

Vitalant Research Institute, San Francisco, USA

Background: A critical barrier to the development of an HIV cure is the lack of an accessible and scalable preclinical animal model that enables robust evaluation of candidate eradication approaches prior to testing in humans. Infection of non-human primates with simian immunodeficiency virus (SIV) is extremely cost prohibitive and labor intensive. A range of humanized mouse models have been developed to investigate HIV persistence. However, these models typically involve engraftment of human fetal tissue which currently faces significant challenges as ethical and political considerations surrounding the use of fetal tissue in scientific research have made it difficult to obtain such material. Driven by this constraint, we sought to establish a model of latent HIV infection that does not involve fetal tissue.

Methods: We transplanted 'J-Lat' cells into 20 irradiated adult NOD. Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice via intravenous injection. J-Lat cells are Jurkat (immortalized) CD4+ T lymphocytes harboring full-length HIV genomes with a frameshift in env and a GFP reporter in place of nef that broadcasts the transcriptional activity of the integrated provirus. 3 weeks post-injection, mice were sacrificed, and single cell suspensions were prepared from various tissues. J-Lat cell engraftment and GFP expression 24 hours post-administration of 20 μg of TNF-α (or vehicle control) were measured using flow cytometry.

Results: J-Lat cells exhibited profound engraftment into several tissue sites within NSG mice, including spleen (mean=0.37%), bone barrow (BM, mean=60.04%) and peripheral blood (PB, mean=6.89%), mirroring the diverse tissue tropism of HIV. Administration of the cytokine TNF-α, an established HIV latency reversal agent, potently induced GFP expression in engrafted cells across tissues (2.5-fold in PB and 3-fold in BM), reflecting viral reactivation. Animals receiving vehicle control did not exhibit any GFP induction.

Conclusions: The data suggest that our 'μ-Lat' murine model of HIV latency enables convenient and efficient testing of 'shock-and-kill' HIV cure agents which are designed to promote clearance of infected cells through the induction of viral expression. In particular, the μ-Lat model will enable us to rigorously determine how effectively viral eradication agents including antiviral gene editing approaches penetrate and function in diverse anatomical sites harboring HIV *in vivo*.

PP 2.7

Donor T cell chimerism correlates with viral reservoir clearance following allogeneic stem cell transplantation in fully cART-suppressed Mauritian cynomolgus macaques

Helen L. Wu[1], Whitney Weber[1], Shaheed A. Abdulhaqq[1], Christine Shriver-Munsch[2], Tonya Swanson[2], Mina Northrup[1,2], Kimberly Armantrout[2], Heidi Price[2], Mitchell Robertson-LeVay[2], Jason S. Reed[1], Katherine B. Bateman[1], Benjamin N. Bimber[2], Stephanie L. Junell[4], Rhonda MacAllister[2], Alfred W. Legasse[2], Michael K. Axthelm[2], Cassandra Moats[2], Jeremy Smedley[2], Theodore R. Hobbs[2], Lauren D. Martin[2], Gabrielle Meyers[3], Richard T. Maziarz[3], Benjamin J. Burwitz[1], Jeffrey J. Stanton[2], Jonah B. Sacha[1,2]

[1] Vaccine and Gene Therapy Institute, [2] Oregon National Primate Research Center, [3] Center for Hematologic Malignancies, Knight Cancer Institute, [4] Division of Medical Physics, Department of Radiation Medicine, Oregon Health and Science University, Portland, Oregon, USA

Background: Two patients remain in ART-free HIV remission following allogeneic hematopoietic stem cell transplant (HSCT) from CCR5-deficient donors, but the mechanisms responsible remain unknown. Here, we examined the impact of fully MHC-matched allogeneic HSCT on SIV reservoir size in ART-suppressed Mauritian cynomolgus macaque recipients.

Methods: Mobilized peripheral stem cells collected from donors by leukapheresis were transplanted into four SIV+, cART-suppressed recipients following reduced intensity conditioning of busulfan, CD3 depletion, and total-body irradiation. Donor engraftment was monitored by Illumina sequencing of single nucleotide polymorphisms.

Recipients were maintained on cART throughout transplantation and follow-up reported here.

Results: Following reduced intensity conditioning and HSCT, SIV DNA cell-associated viral loads (CAVL) in lymph node CD4+ T cells dropped approximately 10-fold in all four SIV-infected, cART-suppressed recipients. HSCT recipients 1, 2, and 3 presented with mixed T cell chimerism and required donor lymphocyte infusions to reach full donor chimerism. Recipient 4 reached 100% donor chimerism in blood T cells by day 200 without donor lymphocyte infusion. In all four recipients, peripheral blood and lymph node CD4+ T cell SIV DNA CAVLs continually decreased as donor chimerism in each compartment increased. At 519 days post-transplant, recipient 4 displayed >99% donor chimerism and undetectable SIV DNA CAVLs in isolated CD4+ T cells from blood, inguinal lymph node, spleen, and bone marrow. In addition, SIV DNA became undetectable in duodenum. Two copies of SIV DNA per million CD4+ T cells were detected in mesenteric lymph node despite 99% donor chimerism in this compartment, raising the possibility that donor cells can become infected during replacement of recipient cells when the protective CCR5 deficient phenotype is absent. Recipient 4 is currently undergoing cART release.

Conclusions: These data suggest that while HSCT immune conditioning decreases the SIV reservoir size, it does not purge the reservoir. Instead, viral reservoir clearance occurs concomitantly with full T cell donor chimerism, suggesting that allogeneic mechanisms contribute significantly to HSCT-mediated HIV cure. We are currently assessing the contribution of CCR5-deficiency to HIV cure using CCR5-blocking antibody Leronlimab in our model of allogeneic HSCT.

PP 2.8

Unprimed CD8 lymphocytes promote the establishment of HIV latency in CD4 T cells

L. Franchitti[1], Z. Zhang[2], J. Yoon[2], M. Paiardini[1], G. Silvestri[1], D.A. Kulpa[2]

[1] Yerkes National Primate Research Center, Department of Pathology and Laboratory Medicine, and Emory Vaccine Center, Atlanta, USA, [2] Department of Pediatrics, Emory University School of Medicine, Atlanta, USA

Background: The persistence of HIV infection under ART is due to a reservoir of latently infected cells that remain indefinitely despite suppression of virus replication. Defining the mechanisms responsible for the establishment and maintenance of the HIV reservoir under ART has been the focus of efforts aimed at HIV eradication. Several studies have demonstrated that $CD8^+$ T cells inhibit virus replication during untreated HIV/SIV infection; however, the mechanisms responsible for this antiviral effect remain poorly understood.

Methods: We used our primary cell based *in vitro* model of HIV latency to study the $CD8^+$ T cell mediated suppression of HIV expression. Memory $CD4^+$ T cells from HIV naïve donors were infected *in vitro* and then co-cultured with activated $CD8^+$ T lymphocytes (1:1 or 1:5 target:effector ratios) in the presence of the anti-retroviral compound saquinavir. After three days, we assessed intracellular Gag expression on $CD4^+$ T cells by flow cytometry, and quantified the frequency of integrated HIV DNA by qPCR. To assess the role of $CD8^+$ T cells in latency reversal, latently infected $CD4^+$ T cells generated in our *in vitro* latency model were TCR stimulated in the presence or absence of activated $CD8^+$ T lymphocytes (1:1 or 1:5 ratios). After three days of activation, we again assessed intracellular Gag expression on $CD4^+$ T cells, and quantified the frequency of integrated HIV DNA.

Results: In the establishment of HIV latency, we found that HIV expression in $CD4^+$ T cells was reduced when co-cultured with $CD8^+$ T cells an average of 9-fold and 18-fold at 1:1 or 1:5 ratios respectively, without significantly reducing the frequency of HIV-infected cells (n=21). We also observed a significant suppression of HIV latency reversal, a 6-fold decrease at 1:1 target: effector ratio and 14-fold decrease at 1:5 ratio.

Conclusions: Our studies demonstrated a $CD8^+$ lymphocyte mediated suppression of HIV expression in $CD4^+$ T cells that functions to induce the establishment as well as maintain latency in the presence of activation signaling. Understanding the mechanisms by which $CD8^+$ lymphocytes suppress virus transcription and ultimately promote HIV latency in ART-treated HIV-infected individuals may provide critical insight to support the design HIV eradication approaches.

PP 2.9

Origin of rebound virus in chronically SIV-infected monkeys following treatment discontinuation

D. Barouch[1], P-T. Liu[1], B. Keele[2], P. Abbink[1], N. Mercado[1], R. Geleziunas[3]

[1] Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA, [2] AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Frederick, USA, [3] Gilead Sciences, Foster City, USA

Background: Viral rebound after antiretroviral treatment (ART) discontinuation in HIV-infected individuals is believed to originate from a small pool of CD4 T cells harboring replication-competent provirus. However, the origin and nature of the rebound virus has remained unclear.

Methods: In this study, we longitudinally evaluated the viral reservoir and the rebound virus in chronically ART-suppressed SIV-infected rhesus monkeys that received Toll-like receptor (TLR) agonists or sham treatment. We sequenced viral RNA and proviral DNA in these animals prior to ART initiation, during ART suppression, and following viral rebound. In particular, we compared near-full length proviral DNA from peripheral blood and lymph node mononuclear cells (PBMCs and LNMCs) during ART suppression with rebound virus after ART discontinuation.

Results: We observed a modest effect of a TLR7 agonist but not a TLR8 agonist on the time to viral rebound. Sequences of the initial rebound virus were generally found in PBMCs and LNMCs harboring structurally intact provirus during ART suppression. Recombinant viruses arose quickly after rebound, but were rare in the initial rebound virus populations.

Conclusions: These data suggest that intact provirus in PBMCs and LNMCs during ART suppression is the likely origin of viral rebound in SIV-infected rhesus monkeys following discontinuation of ART.

PP 2.10

Barcoded HIV reveals effects of PKC modulation on viral reservoir

J. Zack[1], M. Marsden[1], T.H. Zhang[1], Y. Du[1], M. Dimapasoc[1], X. Wu[1], P. Wender[2], R. Sun[1]

[1] UCLA, Los Angeles, USA, [2] UCLA, Stanford, USA

Background: HIV latency is a major barrier preventing cure of the infection in individuals treated with antiretroviral therapy (ART). One suggested strategy for eliminating these latently infected cells is a 'kick and kill' approach, where non-expressing HIV proviruses are induced to produce viral proteins with a latency reversing agent (LRA), allowing them to be killed by viral cytopathic effects, immune effector mechanisms, or other strategies. However, LRAs with entirely optimal safety and efficacy characteristics have yet to be developed, and it is unknown whether LRA administration alone can produce key biologically desired effects including a delay in re-emergence of virus if ART is stopped (viral rebound).

Methods: Here we combine a barcoded HIV approach and a humanized mouse model to study the effects of a synthetic protein kinase C (PKC) modulator LRA on HIV rebound. A phenotypically neutral genetically barcoded HIV swarm was designed, constructed, and validated in vitro, then used to infect humanized bone marrow-liver-thymus mice. Mice were then treated with ART and either dosed with an LRA or vehicle control. ART was stopped 4 days after LRA administration to allow viral rebound and then the rebound timing

and barcode genetic diversity of virus in plasma and spleen virus was quantified and compared between groups.

Results: Administration of the LRA compound during ART resulted in a significant delay in rebound once ART is stopped, and the rebounding virus was composed of a smaller number of unique barcoded viruses than occurs in control-treated animals. This indicates that some reservoir cells present during ART that would have contributed virus to the rebound process can be eliminated by LRA administration.

Conclusions: While a one-time administration of a single LRA is unlikely to eliminate all replication-competent HIV from treated individuals, these data support the continued exploration of 'kick and kill' approaches in HIV cure studies, and provide a pre-clinical model to compare various strategies.

Conflict of interest: Stanford University has filed patent applications on SUW133 and related technology, which has been licensed by Neurotrope BioScience for the treatment of neurological disorders and by Bryologx Inc. for use in HIV/AIDS eradication and cancer immunotherapy. P.A.W. is an adviser to both companies and a cofounder of the latter. J.A.Z. is on the scientific advisory board for BryoLogx, and is a co-founder of CDR3 Therapeutics.

PP 2.11

IL-17A imprints intestinal epithelial cells with the ability to promote HIV-1 dissemination/outgrowth in CD4 T cells

T. Wiche Salinas[1], A. Gosselin[2], B. Mariana[3], H. Touil[1], Y. Zhang[2], J.P. Routy[4], É.A. Cohen[3], C.L. Tremblay[1], P. Ancuta[1]

[1] University of Montreal, Montreal, Canada, [2] CRCHUM, Montreal, Canada, [3] IRCM, Montreal, Canada, [4] McGill University, Montreal, Canada

Background: The crosstalk between intestinal epithelial cells (IEC) and Th17 cells is critical for mucosal homeostasis, with HIV causing major alterations in people living with HIV (PLWH). Mucosal Th17 cells are the first targets of infection and contribute to viral reservoir persistence during antiretroviral therapy (ART). Here, we investigated the effects of IL-17A, a Th17 hallmark cytokine, in regulating HIV dissemination/reactivation in a model of IEC:Th17 interaction in vitro.

Methods: HIV trans infection was studied upon HT-29 IEC activation with IL-17A and/or TNF, exposure to transmitted founder HIV_{THRO}, and co-culture with CD3/CD28-activated memory CD4+ T-cells from uninfected individuals. HIV replication and outgrowth were studied upon co-culture of cytokine-activated IEC with T cells exposed to HIV_{THRO} in vitro and isolated from ART-treated PLWH, respectively. HIV-p24 levels were measured by FACS/ELISA. RNA-sequencing was performed on cytokine-activated IEC before/after co-culture with CD3/CD28-activated T-cells from ART-treated PLWH. Type I IFN was measured using the HEK-blue 293T- cell-based assay.

Results: The ability of IEC to trans infect T-cells was boosted by IL-17A in the presence/absence of TNF. Also, the highest levels of viral replication and outgrowth were observed when T-cells infected with HIV in vitro and isolated from ART-treated PLWH, respectively, were co-cultured with IL-17A-exposed IEC. Exposure of IEC to IL-17A coincided with decreased type I IFN levels in co-cultures. RNA sequencing revealed potential mechanisms for the IL-17A-mediated proviral effects, including decreased expression of transcripts linked to type I IFN production and response (the viral sensor DDX60, the IFN regulatory factor 7, STAT1) and genes relevant for HIV restriction at different steps of the viral cycle (viral entry: IFITM1; disassembly: TRIM5, transcription: IF2AK2, MX1, MX2; translation: IFIT1,3,5, PARP12; assembly: HERC6; release: ISG15, viperin, BST2) and viral RNA degradation (OAS2/3).

Conclusions: In conclusion, IL-17A acts on IEC to create an environment prone to HIV dissemination during primary infection and viral reactivation/outgrowth during the chronic phase, mainly by impairing the type I IFN-mediated antiviral immunity. Thus, new therapeutic interventions aimed at restoring Th17 paucity during ART-treated HIV infection should consider the proviral features of IL-17A.

PP 2.12

Evaluating latency reactivation synergies between the bromodomain inhibitor iBET-151 and the SMAC mimetic AZD5582 in SIV-infected macaques on ART

A.A. Okoye[1], Y. Fukazawa[1], B.E. Randall[1], R. Lum[1], B. Varco-Merth[1], S.D. Falcinelli[2], J. Smedley[1], R. Dunham[2], J.D. Lifson[3], L.J. Picker[1]

[1] Vaccine and Gene Therapy Institute and Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, Or, USA, [2] UNC HIV Cure Center, University of North Carolina at Chapel Hill and HIV Drug Discovery, ViiV Healthcare, Research Triangle Park, Nc, USA, [3] AIDS and Cancer Virus Program, Frederick National Laboratory, Frederick, Md, USA

Background: The Second Mitochondrial-derived Activator of Caspases (SMAC) mimetic AZD5582 has recently been shown to be an effective HIV/SIV latency reversing agent (LRA) in vivo. Preliminary studies also indicate a potent synergistic effect when bromodomain inhibitors are used in combination with SMAC mimetics to reverse HIV latency ex vivo in CD4+ T cells from HIV+ individuals on ART. Here, we evaluated whether the bromodomain inhibitor iBET-151 can synergize with AZD5582 to induce SIV production in SIV-infected rhesus macaques (RM) on suppressive ART.

Methods: A total of 4 RM were intravenously (IV) inoculated with SIVmac239 and placed on ART (tenofovir/emtricitabine/dolutegravir) 56 days later. After virus suppression to <15 RNA copies/ml plasma, 2 RM received 8 weekly, IV injections of AZD5582 in combination with iBET-151 (administered orally, 3 times a week) followed by a further 8 weekly injections of AZD5582. The other 2 RM received 16 weekly injections of AZD5582 alone. Cell associated SIV DNA, RNA and plasma SIV RNA were quantified by qPCR and qRT-PCR, and lymphocyte population dynamics assessed by flow cytometry.

Results: AZD5582 +/– iBET-151 induced significant up-regulation in the interferon-inducible surface protein CD169 on CD14+ monocytes and increases in proliferating CD4+ and CD8+ memory T cells by Ki67 expression and in blood. However, no increase in T cell activation, as measured by CD69 expression, was observed. All RM also showed 1-2 log increases in pvl over the treatment period, which returned to baseline levels after treatment was stopped. Interestingly, RM treated with AZD5582 alone showed increases in SIV RNA/DNA ratio (> 6 times after 8th dose of SMAC) and decreases SIV DNA (> 3 times) in PBMC over time. Preliminarily, we have not observed enhancement of AZD5582 activity with the addition of iBET-151 in this pilot regimen.

Conclusions: While this pilot study indicates no synergistic effect of iBET-151 with AZD5582, these data confirm previous observations demonstrating AZD5582 is a potent LRA which can be used to induce SIV production in vivo in non-human primate HIV cure studies. Further studies are under consideration to refine the dosing of iBET-151 to better understand synergic effect of iBET-151 and AZD5582.

Conflict of interest: R. Dunham is an employee of ViiV Healthcare.

PP 2.13

Next generation sequencing in a direct infection model reveals important parallels to *in vivo* reservoir dynamics

U. O'Doherty[1], M. Pinzone[1], M.P. Bertuccio[2], D.J. Vanbelzen[3]

[1] University of Pennsylvania, Philadelphia, USA, [2] University of Messina, Messina, Italy, [3] Northwestern University, Evanston, USA

Background: While in vitro models do not recapitulate in vivo infection, they can provide powerful insights. We used Next Generation Sequencing (NGS) to address how the metabolic state affects reservoir formation and dynamics. The pace of infection is accelerated in metabolically active cells, but these cells are prone to spreading infection and high turnover. On the other hand, directly infected resting cells may be a strong model for latency but their reverse

transcripts are likely to contain deletions so intact proviruses may form rarely. These deletions may form due to the presence of restriction factors and lower levels of deoxynucleotides (dNTPs), resulting in more pausing during reverse transcription.

Methods: We sequenced ~1000 HIV proviruses at 2, 4 and 8 days after NL4-3 infection of metabolically active and quiescent CD4 T cells (2 ng/ml IL-7) cultured with saquinavir to prevent spreading infection. We isolated integrated and total HIV DNA by pulsed-field electrophoresis before performing sequencing.

Results: As predicted, reverse transcripts with massive deletions formed more frequently in resting CD4 T cells (Fig. 1A). Surprisingly, the vast majority of proviruses were replication-competent in both resting and activated CD4 T cells after a single-round infection (Fig. 1B). Thus, we infer that the majority of deleted reverse transcripts do not integrate into the human genome. This in turn suggests that positive selection for deleted proviruses in vivo must be strong and separating integrated HIV DNA before NGS is critical to understand selection pressures on integrated proviruses. Consistent with in vivo modeling, the decay kinetics were similar in metabolically resting and activated cells. Decay of intact proviruses was faster than deleted forms demonstrating another setting where this model recapitulates reservoir dynamics.

Conclusions: Sequencing HIV DNA longitudinally after direct infection of metabolically quiescent and activated T cells provides a model that recapitulates important aspects of reservoir decay. While reservoir decay is accelerated in vitro, we envision our model could be a powerful tool for preclinical testing. Moreover, our Results: suggest in some clinical scenarios it would be important to separate integrated from unintegrated HIV DNA to study selection pressures.

PP 2.14

Treatment of SHIV-infected, ART-suppressed rhesus macaques with bispecific HIVxCD3 DART® molecules

Daniel M. Gorman[1], Ming-Tain Lai[2], Carolyn McHale[2], Meiqing Lu[2], Don Graham[2], Julie Strizki[2], Yanyan Zheng[1], Shirley Ma[1], Soha Moltagh[1], Marc Bailly[1], SuChun Hseih[1], Glareh Azadi[1], Yaoli Song[1], Fernando Ugarte[1], Romina Riener[1], Wendy Blumenschein[1], Daria Hazuda[2], Jeffery Nordstrom[3], Bonnie Howell[2]

[1] Merck, South San Francisco, CA, USA, [2] Merck, West Point, PA, USA, [3] Macrogenics, Rockville MD, USA

Background: New strategies are needed to eliminate HIV viral reservoirs, diminish need for ART and restore a robust immune system. Multispecific antibodies that redirect T cells to recognize and kill antigen-expressing target cells is one approach. Bispecific antibodies targeting CD19 on B-cell lymphoma and CD3 on effector T-cells have achieved clinical success. We evaluated bispecific HIVxCD3 DART molecules targeting HIV-1 envelope and CD3 in SHIV-infected rhesus macaques (RM) maintained on ART to determine effect on viral reservoir and delay to viral rebound after analytical ART interruption (ATI).

Methods: 25 RMs were infected with SHIV-SF162P3 and ART initiated at 6 months. After 2 months, RMs received 6 weekly doses of 1 mg/kg HIVxCD3 DART molecules with anti-HIV envelope arms derived from nonneutralizing mAbs specific for gp120 C1/C2 or gp41 (A32 or 7B2 DART molecules, respectively) or RSVxCD3 control molecule. ATI was 1 week after the last DART molecule dose. Peripheral blood, lymph node, and rectal biopsies were collected longitudinally. Viral loads, drug PK and ADA, cytokine levels, blood cell enumeration and phenotyping, T-cell binding and whole blood gene expression profiling were measured.

Results: DART molecule treatment was well-tolerated, PK was comparable across groups, and target engagement of CD3 on CD4 and CD8 T cells was demonstrated. Enhanced T cell activation (CD69 and PD-1) was observed in a subset of RMs following DART molecule administration and type I IFN-related inflammatory gene signature was identified in PBMCs across all groups. ADA was detected after completion of dosing in 2-3 animals per group. There was no notable effect on plasma viral load or cell-associated viral measures in tissues or PBMCs among the groups. Viral load rebounded ~ 7-14 days post-ATI but was delayed until 42 days in one A32 DART molecule-treated RM.

Conclusions: Treatment of SHIV-infected rhesus monkeys with A32 or 7B2 DART molecules, while maintained on suppressive ART, had minimal or no inhibitory effect on viral rebound upon subsequent ART interruption. The general lack of evidence for HIVxCD3 DART molecule-mediated anti-viral activity is consistent with low availability of envelope-expressing, virus-infected target cells in SHIV-infected RMs maintained on ART suppression.

PP 2.15

Single-cell transcriptome of in vivo SIV-infected rhesus macaque CD4 T cells

A. Tokarev[1,2], A. Geretz[1], P. Ehrenberg[2], M. Roederer[3], R. Thomas[1], D.L. Bolton[1]

[1] Henry Jackson Foundation for the Advancement of Military Medicine, Bethesda, USA, [2] US Military HIV Research Program, Silver Spring, USA, [3] Vaccine Research Center, NIAID, NIH, Bethesda, USA

Background: Host genes differentially expressed during cellular HIV-1/SIV infection may promote viral replication and persistence, and thus represent potential drug targets. However, the transcriptional program of infected cells in vivo is poorly defined, due to challenges in their isolation.

Methods: Lymph node memory CD4 T cells (N=752) from an SIVmac251-infected rhesus macaque 10 days post-infection were phenotyped and isolated by single-cell FACS and RNA was sequenced using the SMART-seq technology on the Illumina NovaSeq Platform. Sequencing reads were aligned to the Macaca mulatta and SIVmac251 reference genomes (Genbank release 0.0.1). Spliced and unspliced mRNA were examined using the Miso framework.

Results: Sequencing reads aligning to the SIV reference genome were used to identify SIV-infected cells and define their stage in the viral life cycle. SIV RNA+ cells (N=287) exhibited an SIV read count distribution of 1-800,000 (median ~4,600). Spliced mRNAs were used to distinguish transcriptionally active infected cells, defined as double positive for splice junctions found in tat/rev- (D4-A7) and env- (D1-A5) encoding transcripts (N=117). The median SIV RNA content for these cells was 10.3% of the transcriptome (IQR 6.3-15.4%; percent of total reads). SIV RNA+ cells deemed less likely to be productively infected, lacking both of these spliced RNA markers and <1% of reads mapping to SIV (N=156), contained a median SIV RNA content of 0.05%. Surface expression of both CD3 and CD4 was diminished on transcriptionally active infected cells compared to SIV RNA-negative (N=465) and SIV RNA+, spliced mRNA-negative cells (both $p<0.0001$). The transcriptionally active infected cells expressed higher surface levels of activation markers while expression of germinal center homing marker CXCR5 did not differ.

Conclusions: Viral transcriptome analysis of in vivo SIV-infected CD4 T cells revealed a highly transcriptionally active state consistent with productive infection. Host proteins and genes identified as differentially regulated in these cells may support virus replication and persistence, and thus represent potential drug targets for prophylactic and therapeutic strategies.

PP 2.16

Novel SHIVs encoding transmitted/founder Envs for latency and cure research

A. Bauer[1], E. Lindemuth[1], L. Kuri-Cervantes[1], H. Li[1], M. Watkins[2], W. Ziani[2], H. Xu[2], R. Veazey[2], G. Shaw[1], K. Bar[1]

[1] University of Pennsylvania, Philadelphia, USA, [2] TNPRC, Covington, USA

Background: A robust simian-human immunodeficiency virus (SHIV)-macaque model of HIV-1 latency is critical to investigate eradicative

and suppressive strategies that engage Env. We have generated >20 SHIVs encoding transmitted/founder (TF) or primary HIV-1 Envs, all of which successfully infect rhesus macaques (RM). Here, we evaluate two TF SHIVs, SHIV.D.191859 and SHIV.C.CH848, hereafter referred to as SHIV.D and SHIV.C, which encode TF subtype D and C HIV-1 Envs respectively, for their viral kinetics and persistence during combination antiretroviral therapy (cART) and analytical treatment interruption (ATI) in rhesus macaques (RM).

Methods: 6 RM were mucosally or IV inoculated with SHIV.D; 4 additional RM were IV inoculated with SHIV.C. Viral kinetics through the establishment of peak and setpoint viremia, 24 weeks of cART, and ATI were assessed via plasma RT-PCR. Single genome sequencing of plasma virus was used to characterize the diversity of pre-cART and rebounding viruses. Ca-DNA and ca-RNA in PBMC was quantified during chronic infection and after 12 weeks of cART.

Results: Inoculation of 6 RM with SHIV.D led to high peak (10^{5-8} c/ml) and set point (10^{3-6} c/ml) viral loads (VL) in all RM for at least 42 weeks post-infection (WPI). At between 6 and 18 months of infection, 4 RM with high setpoint viremia (10^{3-6} c/ml) were placed on cART for 24 weeks. Viral suppression was achieved in all RM 7-21 days post-cART initiation. Viral rebound occurred between 7-29 days post-ATI in all 4 RM. Inoculation of 4 RM with SHIV.C led to high peak (10^{5-7} c/ml) and set point (10^{3-5} c/ml) VL. At 16 WPI, the 4 RM were placed on cART. Viral suppression was rapidly achieved and maintained for 24 weeks. Viral rebound occurred between 12-29 days post-ATI. Sequencing of rebound plasma vRNA revealed multiple genetically distinct virus populations at first detectable rebound in 7 of 8 SHIV.D and C-infected RM. Notably, both ca-DNA and RNA remained detectable in all 8 SHIV.D and C-infected RM after 12 weeks of cART.

Conclusions: The viral dynamics and reservoir biology before, during, and upon cART interruption make SHIV.D and SHIV.C promising reagents for a SHIV model of HIV-1 latency and cure.

Session 3: Virology of HIV persistence

OP 3.1

Differential decay of intact and defective proviral DNA in HIV-1-infected individuals on suppressive antiretroviral therapy

M. Peluso[1], P. Bacchetti[1], K. Ritter[2], S. Beg[3], P. Hunt[1], T. Henrich[1], J. Siliciano[3], R. Siliciano [3], G. Laird[2], S. Deeks[1]

[1] University of California, San Francisco, San Francisco, USA, [2] Accelevir Diagnostics, Baltimore, USA, [3] Johns Hopkins School of Medicine, Baltimore, USA

Background: The latent HIV-1 reservoir is established early in the course of infection and persists despite suppressive antiretroviral therapy (ART). The relative stabilities of the intact and defective HIV genomes over time during effective ART have not been fully characterized. Understanding variability in the rate of change of the reservoir size, correlates of this variability, and factors associated with rapid decay is likely to be useful in the design and interpretation of HIV cure interventions.

Methods: We used the intact proviral DNA assay (IPDA) to estimate the rate of change of intact and defective proviruses in HIV-infected adults on suppressive ART over several years. We used linear spline models with a knot at seven years; these included a random intercept and slope up to the knot. We also estimated the influence of covariates on levels at the start of suppression and rates of change.

Results: We studied 81 individuals for a median of 7.3 (IQR 5.9-9.6) years. In a model allowing for a change in the rate of decline, we found evidence for more rapid declines in intact genomes from initial suppression through seven years (16.0% per year decline; CI -23.0%, -8.4%) followed by a slower rate (3.6% per year; CI -8.1%, +1.1%). The estimated half-life of the reservoir was 4.0 years (CI 2.6-7.9) until year seven and 19.0 years (CI 8.2-infinite) thereafter. Intact provirus declined at a faster rate than defective provirus ($p<0.001$). There was substantial variability between individuals in the rate of decline until year seven. In multivariate models, individuals with higher CD4+ T-cell count nadir values had a faster rate of decline. A subset of individuals (n=7) were estimated to have very rapid declines (>30% per year).

Conclusions: These Results: demonstrate a non-linear decay of viral genomes over time. Intact proviral genomes decay more rapidly than defective ones. The mechanism for this difference is not clear, but could involve cells with intact genomes experiencing increased cytopathic effects or enhanced immune targeting due to virus protein production. These findings provide evidence that the biology of the replication-competent (intact) reservoir differs from that of the replication-incompetent (non-intact) pool of proviruses.

OP 3.2

Women undergoing reproductive aging show increased reservoir sizes associated with removal of hormonal control of HIV-1 latency by oestrogen

C. Dobrowolski[1], J. Karn[1], E. Scully[2], K.M. Weber[3], A.L. Landay[4]

[1] Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, USA, [2] Johns Hopkins University, Department of Medicine, Division of Infectious Diseases, Baltimore, USA, [3] WIHS/CORE Center of Cook County Health, Chicago, USA, [4] Rush University Medical Center, Department Internal Medicine, Chicago, USA

Background: The estrogen receptor-α (ESR-1) is a potent repressor of HIV. HIV emergence from the latent reservoir can be repressed by estrogen and other ESR-1 agonists, and suppressed by Selective Estrogen Receptor Modulators (SERMS), which are antagonists to ESR-1.

Methods: Leukapheresis samples from a cohort of 12 well-matched reproductive age women and men on fully suppressive ART were evaluated by the EDITS assay measuring production of spliced envelope (env) mRNA by next generation sequencing. EDITS assays were also used to assess the impact of hormonal changes after menopause using carefully staged longitudinal samples from 15 women obtained from the Chicago WIHS cohort.

Results: The patient cells were activated by TCR stimulation, IL-15 or SAHA in the presence of either β-estradiol or a SERM. Although both sexes responded to SERMs and β-estradiol, females showed much higher levels of inhibition in response to the hormone and higher reactivity in response to ESR-1 modulators than males. Importantly, the total inducible RNA reservoir, as measured by the EDITS assay, was significantly smaller in the women than in the men. Remarkably, estrogen nearly completely blocked viral spread in females, but not in males. Consistent with these Results:, women who are undergoing menopause show progressive increases in their RNA-inducible reservoir sizes.

Conclusions: ESR-1 is a pharmacologically attractive target that can be exploited in the design of therapeutic strategies for latency reversal. This work provided the scientific underpinning for a preliminary trial of the effects of Tamoxifen and vorinostat on the HIV reservoir among postmenopausal HIV-infected women (ACTG A5366, ClinicalTrials.gov Identifier: NCT03382834). Induction of HIV RNA after vorinostat by the EDITS assay was primarily seen among women with increases in histone acetylation but there were no additional effects of tamoxifen. Concurrent exposure to estrogen is likely to limit the efficacy of viral emergence from latency and it is striking that reservoir sizes in women are smaller than in men. Progression through stages of reproductive aging, when estrogen levels fall, is predictive of expansion of the inducible RNA reservoir.

OP 3.3

Contribution of antigenic exposure to the persistence of HIV-infected CD4 T cells *in vivo*

F. Simonetti[1], H. Zhang[1], G. Soroosh[1], S. Beg[1], H. Raymond[2], K. Mccormick[2], S. Deeks[3], F. Bushman[2], J. Siliciano[4], R. Siliciano[1]

[1] Johns Hopkins University, Baltimore, USA, [2] University of Pennsylvania, Philadelphia, USA, [3] University of California San Francisco, San Francisco, USA, [4] Johns Hoppkins University, Baltimore, USA

Background: Proliferation of infected CD4+ T-cells represents a major mechanism of HIV persistence. However, it is unclear to which extent this phenomenon is the result of homeostatic proliferation, response to antigens, or effects related to the site of HIV integration. We hypothesized that clonal selection, driven by recurrent antigens, contributes to the persistence of HIV-infected cells.

Methods: We studied 10 HIV+ donors on ART who were co-infected with CMV. PBMC were briefly stimulated with CMV lysates, Gag peptides or αCD3/28 antibodies. We sorted responding (CD40L+CD69+) and non-responding memory (CD40L-CD69-CD45ROhi) CD4+ T-cells. HIV-infected and global CD4+ T-cell populations were analyzed by proviral single genome sequencing and TCRβ immuno-sequencing, respectively. To characterize proviruses in Ag-specific clones, we used whole genome amplification on small pools of cells at limiting dilution and performed integration site analysis and full-length proviral sequencing. A viral outgrowth assay (VOA) was used in one donor to identify infectious proviruses.

Results: PBMC stimulation allowed us to access the expected frequencies of CMV- and Gag-specific CD4+ T-cells (mean 2.8% and 1.1%, respectively). Compared to cells responding to non-specific CD3/28 stimulation, proviruses in CMV-specific cells had a higher proportion of identical sequences (0.73 vs 0.28, $p<0.0001$) and higher clonality (mean Gini 0.6 vs 0.2, $p=0.0002$). Identical proviruses in Gag-specific cells were common across individuals, but less dominant. Similarly, TCRβ analysis showed a higher clonality in CMV-specific cells compared to Gag-specific and non-responding memory cells (mean clonality 0.2 vs 0.05 vs 0.03, respectively). Identical proviruses were detected at multiple time points and were confirmed by integration site analysis. Some clones showed integration in BACH2, STAT5B and MKL1. Most of these clones carried defective proviruses. However, in one individual, we identified the same VOA isolate in four wells from CMV-specific cells that matched multiple DNA sequences from CMV-specific cells collected 8 months

previously, suggesting the persistence of a CD4+ clone in response to CMV antigens that harbored an infectious provirus.

Conclusions: We provide direct in vivo evidence that proliferation of HIV-infected cells is common for CMV- and Gag-specific CD4+ T-cells, demonstrating that antigenic pressure represents a selective force shaping the fate of both defective and replication-competent proviruses.

OP 3.4

Multiplexed RNA flow cytometric FISH allows single-cell viral transcriptional profiling and phenotypic characterisation of translation-incompetent HIV reservoirs

D. Kaufmann[1], M. Dubé[1], G. Sannier[1], N. Brassard[1], G.G. Delgado[1], A. Baxter[1], J.P. Routy[2], N. Chomont[1]

[1] Research Centre of the Centre Hospitalier de l'Université de Montréal and Université de Montreal, Montreal, Quebec, Canada, Montreal, Canada, [2] Chronic Viral Illnesses Service and Division of Hematology, McGill University Health Centre, Montreal, Canada

Background: Evidence suggests that viral reservoirs (VR) during ART correspond to cell populations that are highly heterogeneous in their potential to progress from latent infection to transcription of viral genes, to translation of viral proteins, and to productive infection. A better understanding of this broad VR diversity is key to develop cure strategies, but its study at the single-cell level in primary human samples is very challenging.

Methods: We developed a multiplexed RNA flow cytometric fluorescent in situ hybridization (RNA flow-FISH) assay for the simultaneous detection of three viral RNAs, along with p24 staining and phenotyping. We conducted an observational study on 11 viremic HIV-infected persons prior to ART (UNT), 10 suppressed people (ART) and 11 uninfected controls (UC). We examined CD4 T cells isolated from PBMC. Data were analyzed by FlowJo and Prism.

Results: This approach allows versatile combinations of RNA probes to identify transcriptional co-expression patterns of viral genes at the single-cell level and discriminate between VR cells that do or do not express HIV proteins (e.g, vRNA+ p24+ vs vRNA+p24-). HIV-infected cell identification by vRNA co-expression was highly sensitive and specific, with a false-positive rate <3/vRNA+ events/10^6 in UC. In UNT, the majority of vRNA+ cells expressed p24+; this fraction was stable (median [interquartile range] p24+=73% [65%; 76%]), consistent with ongoing viral replication. In contrast, PMA/ionomycin stimulation in ART lead to induction of a vRNA+ VR (median [IQR] vRNA+ CD4=75 [16; 123]/10^6), only a small fraction of which expressed p24, this with large interindividual differences (median[IQR] p24+=7% [5%; 9%]).

Conclusions: Multiplexed RNAflow-FISH bridges the gap between cytometric and PCR-based detection methods. It reveals an inducible vRNA+ VR substantially larger than some previous estimates and provides a high-throughput single-cell method to characterize VR irrespectively of their capacity to express viral proteins. This versatility will ease VR monitoring and lead to a better comprehension of their dynamics in vivo, including their potential for recognition by effector arms of the immune system.

OP 3.5

Low viral reservoir treated individuals show unusual HIV latency distribution

C. Gálvez[1], V. Urrea[1], S. Benet[1], B. Mothe[1], L. Bailón[2], J. Dalmau[1], L. Leal[3], F. García[3], J. Martinez-Picado[1], M. Salgado[1]

[1] AIDS Research Institute IrsiCaixa, Badalona, Spain, [2] Infectious Diseases Department, University Hospital 'Germans Trias i Pujol', Badalona, Spain, [3] Infectious Diseases Department, Hospital Clínic, University of Barcelona, Barcelona, Spain

Background: Small-size viral reservoirs are predominantly found in HIV-1 controllers and individuals treated during acute/early HIV-1 infection. However, other HIV$^+$ subjects could naturally also harbor low viral reservoirs. We have established a cohort of 'Low Viral Reservoir Treated' subjects (LoViReT) to further explore the mechanisms associated with low reservoir levels.

Methods: 42 HIV$^+$ individuals under cART and <50 HIV-DNA copies/10^6 PBMCs constitute the LoViReT cohort; at least 66% of them initiated cART during the chronic phase of HIV-1 (>6 months since acquisition). In 12 LoViReTs, total HIV-DNA was longitudinally measured in cryopreserved CD4$^+$ T-cells by ddPCR, including a pre-cART sample. 14 LoViReTs underwent a leukapheresis to measure replication-competent virus by qVOA (37x10^6 CD4$^+$T-cells), and total HIV-DNA in sorted CD4$^+$T-cell subsets. In 9 LoViReTs with <0.1 infectious units per million (IUPM), total HIV-DNA was measured in rectal and/or lymph node biopsies (LN). Clinically matched individuals with HIV-DNA >50 HIV-DNA copies/10^6 PBMCs were recruited as controls.

Results: LoViReTs harbored significantly lower total HIV-DNA in CD4$^+$T-cells before cART initiation compared to controls (1,051 and 5,995 HIV-DNA copies/10^6 CD4$^+$T-cells respectively, P=0.002) despite comparable pre-cART viral load. These differences became higher after 5 years on cART (16 vs. 5-folds decay respectively, P<0.001). 10/14 LoViReTs had undetectable replication-competent virus (IUPM<0.0185) >10 years after cART. Among them, we detected low levels of HIV-DNA in rectum in 6/8 subjects with a median of 57 HIV-DNA copies/10^6 CD45$^+$ T-cells [IQR:37-114]. In LN, only 3/8 subjects had detectable reservoir (263 [IQR:36-2,112] HIV-DNA copies/10^6 CD45RA$^-$ T-cells). Unexpected HIV-reservoir distribution was observed in LoViReTs, being short-live transitional memory (T_{TM}) and effector memory (T_{EM}) T-cells the major contributors to the total reservoir (47% and 29% respectively). T_{CM} presented limited contribution to the HIV reservoir (24%).

Conclusions: LoViReT individuals have abnormally low HIV-reservoirs before cART initiation. 71% of LoViReTs did not have replication-competent virus and harbored limited provirus in tissue sanctuaries after a median of 15 years under cART. A cause of this exceptional low reservoir could be the high contribution of the short-live T_{TM} and T_{EM} cells in the HIV reservoir. This unique group of individuals are of great interest as trial participants in eradication studies.

PP 3.1

Automated high-throughput quantification of persistent HIV-1 plasma viremia in individuals on ART

J. Jacobs[1], M. Tosiano[1], D. Koontz[1], A. Worlock[2], K. Harrington[2], K. Shutt[1], S. Bakkour[3], M. Busch[3], J. Mellors[1]

[1] University of Pittsburgh School of Medicine, Department of Medicine, Division of Infectious Diseases, Pittsburgh, USA, [2] Hologic, Inc., San Diego, USA, [3] Vitalant Research Institute, San Francisco, USA

Background: Low-level plasma HIV-1 viremia persists in the majority of HIV-1 positive individuals despite long-term clinically-effective ART. Clearance of HIV-1 viremia remains a critical goal towards an HIV cure, but complex and low-throughput single copy assays (SCA) limit the capacity to monitor the effects of interventions on persistent viremia. Here we report the evaluation of two high-throughput methods on the Hologic Panther platform to automate quantitation of low-level viremia in comparison with a SCA targeting integrase (iSCA2.0; Tosiano, et al. J Clin Micro 2019).

Methods: The assay methods performed on the Hologic Panther platform were: 1) testing of nine 0.5mL replicates (Panther 9x) with estimation of HIV-1 RNA concentration using statistical inference based on binary outcome; and 2) concentration of 5mL plasma to one 0.7 mL replicate by centrifugation (Panther spun). Plasma HIV-1 RNA standards (20, 5, 2.5, 1.25, 0.625, and 0 copies/ml) from the Quality Assurance (VQA) at Rush University were tested in 5 independent runs of 5 replicates. Both Panther methods were compared to the manual iSCA 2.0. Mean, standard deviation and percent positive assays were calculated for each run and the 95% LOD was assessed using maximum likelihood estimation.

Results: Assay Results: are summarized in the Table. The 95% LODs (95% CI) were 2.3 (1.6, 3), 3.0 (2.1, 3.8), 3.9 (2.8, 5) for iSCA2.0,

Panther 9x and Panther spun, respectively, indicating that iSCA2.0 was most sensitive but that Panther 9x was only marginally less sensitive. Panther spun had reduced sensitivity compared to the other methods. Each assay had 100% specificity across 25 replicates of 0 copies/ml. The weekly estimated throughput for the Panther 9x method is 5-10 times that of iSCA 2.0.

Conclusions: Conclusions: Although the manual single copy assay targeting HIV-1 integrase (iSCA 2.0) has the lowest 95% limit of detection for plasma HIV-1 RNA, multiple replicate testing (9x) on the Hologic Panther platform has similar sensitivity and could be used as a screening tool for higher throughput monitoring in clinical trials of interventions aimed at clearing persistent viremia towards a functional cure of HIV-1 infection.

PP 3.2

Proviral landscape in children parallels adults and enables reservoir reconstruction

J. Hasson[1], M.G. Katusiime[2], S. Smith[3], M. Cotton[2], E. Boritz[3], J. Coffin[4], J. Mellors[5], S. Patro[1], G. Van Zyl[2], M. Kearney[1]

[1] National Cancer Institute, Frederick, USA, [2] Stellenbosch University, Cape Town, South Africa, [3] National Institute of Allergy and Infectious Diseases, Bethesda, USA, [4] Tufts University, Boston, USA, [5] University of Pittsburgh, Pittsburgh, USA

Background: Characterizing HIV-1 proviruses that lead to viral rebound upon ART interruption could inform strategies for a functional cure. Methods for measuring the HIV-1 reservoir require collecting large sample volumes that are difficult to obtain from children. Here, we profile the proviral landscape in children and demonstrate the utility of 'viral reconstruction' to characterize the genetics of the HIV-1 reservoir when proviral copy numbers are low.

Methods: We performed near-full length (NFL) single-genome sequencing on 210 amplicons from PBMC of two children treated early (9 months) and on ART for 7 years. The proviral landscape was compared to that of adults on ART (1056 genomes in the Proviral Sequence Database). Because recovering intact proviruses is rare in children and in adults who initiate ART early, we used the population of defective proviruses to reconstruct NFL ancestors that may be similar to the founder virus and/or to intact proviruses that persist on ART.

Results: Similar to adults, ~98% of the proviruses were defective including 60% with large 3′ deletions of env/tat/rev. Proviral diversity (0.3% and 0.7% in p6-PR-RT) and proviral copy number (47 and 182 copies/106 PBMC) were low. In the child with the lower diversity and fewer 3′ deletions, we identified defective proviruses with sequences identical except for non-overlapping deletions, allowing reconstruction of the ancestor that infected these cells and is likely similar to both the founder virus and reservoir proviruses. Indeed, the reconstructed virus matched an intact provirus from the same sample, demonstrating the accuracy of the approach.

Conclusions: Despite very different immune systems, the HIV-1 proviral landscapes on ART were not obviously different between children and adults, with most proviruses containing large 3′ deletions. Few infected cells in children and in early-treated adults makes it difficult to detect intact proviruses. We demonstrate the utility of viral reconstruction to infer the genetics of possible transmitted founder viruses and of intact proviruses that may comprise the reservoir. Characterizing the genetics of the reservoir in early-treated individuals can help guide the design of therapeutic interventions for HIV remission.

PP 3.3

The dynamics of HIV-1 quasispecies diversity circulating in the plasma RNA and cellular DNA of patients with ART

M. Liying[1], Z. Yuanyuan[2], Y. Qianqian[2], N. Ming[3], L. Tingting[1], W. Chen[2], D. Yibo[2], L. Lingjie[2], X. Hui[2], C. Chen[1]

[1] Institute of Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [2] State Key Laboratory of Infectious Disease Prevention and Control, National Center for AIDS/STD Control and Prevention, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Chinese Center for Disease Control and Prevention, Beijing, China, [3] Beijing Institute of Radiation Medicine, Beijing, China

Background: Study on intra-host HIV quasispecies diversity and evolutionary pattern will provides insights into the mechanisms of viral pathogenesis, persistence, and virologic failure on antiretroviral therapy (ART).

Methods: Here we show the dynamics of HIV-1 quasispecies diversity circulating in the plasma RNA and cellular DNA of patients with ART by means of ultra-deep sequencing and phylogenetic analysis.

Results: The Results: of this studies exhibited that ~ 60% quasispecies diversity is consistent in HIV RNA and DNA. Analysis of mutations distribution (iSNV and SNP) and mutated allele frequency reveal that the mutations with higher frequency have more chance to participate into the HIV life cycles. We show a landscape of the mutations in total cellular HIV DNA after long-term ART. And, during the ART, drug-resistant associated mutations (DRAM) were accumulated and the ratio of DRAMs to all mutations dramatically increased either in treatment failure patients or in virologic failure patients.

Conclusions: Our result provides a new sight to clarify the intra-host HIV quasispecies diversity and dynamics under ART. HIV DNA, as a part of HIV nucleic acid, can be regarded as a potential target for detection and surveillance the emerge drug-resistant HIV.

PP 3.4

High-throughput sequencing of integrated HIV-1 reveals novel proviral structures

K. Joseph[1], E. Halvas[1], L. Brandt[1], S. Patro[2], J. Rausch[3], M. Kearney[2], J. Coffin[4], J. Mellors[1]

[1] University of Pittsburgh, Department of Medicine, Division of Infectious Disease, Pittsburgh, Pa, USA, [2] HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD, USA, [3] Basic Research Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD, USA, [4] Tufts University, Boston, MA, USA

Background: Efforts to cure HIV-1 infection will require a better understanding of the HIV-1 reservoir but characterizing individual integrated proviruses has remained difficult because of technical challenges related to the rarity of proviruses in CD4+ T-cells. Current approaches for sequencing integration sites using NGS are inefficient (most reads are off-target) and restricted read lengths can make it difficult to identify both integration sites and proviral sequences.

Methods: We have developed a new automated approach that sequences single HIV proviruses and their 5′ host integration sites by: i) amplifying the whole cellular genome at a proviral end point through multiple displacement amplification; ii) performing PCR that amplifies variable and near-full length proviruses; and iii) performing nullomer-mediated PCR (a new type of PCR using a linker consisting of nullomer motifs absent in target genomes), which markedly enhances specificity for integrated proviral targets. Amplicons can be sequenced by dideoxy (e.g., Sanger) and/or NGS methods.

Results: Amplicons sequenced by NGS utilized >90% of reads on average during consensus generation for both proviral and integration site amplicons. The workflow sequences all but 69 bp of the 3′ LTR of the provirus. Across 5 donors, an average of 78% of HIV-positive MDA reactions yielded the 5′-host-virus junction containing 400 + 297 bp of flanking host sequence and 13.4% of proviruses were near-full length (n=247 proviruses total). To date the assay has been used to characterize a broad range of both intact and defective integrated proviruses in blood mononuclear cells from donors on suppressive ART including replication-competent proviruses in cell clones (proven by viral outgrowth) and novel proviral structures such as asymmetrical LTR deletions and genome inversions (Figure). The accuracy of the method has been confirmed by sequence identity with full-length and deleted proviruses amplified directly from blood mononuclear cells using integration site-specific primers.

Conclusions: This novel integrated proviral sequencing assay provides an efficient and high-throughput means of characterizing proviral reservoirs that need to be targeted to achieve a cure of HIV-1 infection.

PP 3.5

HIV-infected cells that survive co-culture with HIV-specific CTL exhibit distinctive viral messenger RNA transcript profiles

R.B. Jones, G. Lee, T. Klevorn, Y. Ren

Weill Cornell Medical College, New York, USA

Background: The *in vitro* co-culture of productively HIV-infected cells with functional cytotoxic T cells (CTL) typically Results: in reductions in infected-cell frequencies, but not in the eradication of infections. We postulate that surviving cells may possess unique features that enable their persistence. Here, we interrogate viral transcriptional profiles of these survivors in comparison to infected cells that were not exposed to CTL.

Methods: PBMC-derived memory $CD4^+$ T cells from an antiretroviral-treated HIV-infected individual were superinfected with HIV JR-CSF or left uninfected, then cultured overnight with antiretrovirals with or without an autologous Gag-specific (IK9) CTL clone targeting the IRL-RPGGKK epitope at an effector-to-target ratio of 2:1. Cells were sorted into $CD3^+$,$CD8^-$,$CD4^-$,Gag^+ and $CD3^+$,$CD8^-$,Gag^- populations. Bulk RNAseq was performed on all five populations (CTL^+Gag^+, CTL^-Gag^+, CTL^-Gag^+, CTL^-Gag^-, no-superinfection control) using Illumina TruSeq non-stranded poly-A library preparation kit and HiSeq4000 with read length of 100 base pairs. Viral transcripts were bioinformatically captured using an in-house R pipeline. Chi-square tests with Yates' correction were used for statistical comparisons.

Results: In all five conditions, we were able to detect HIV transcripts that mapped to all genomic regions of the virus including *gag, pol, vif, vpr, vpu, env, nef, tat* and *rev*. As expected, in the absence of CTL, Gag^+ cells had higher abundance of viral transcripts than Gag^- cells (CTL^-Gag^+ 7% versus CTL^-Gag^- 0.2%, $p<0.0001$, Fig1). CTL co-culture reduced infected (Gag^+) cells frequency from 35% to 17%. Interestingly, the surviving infected cells exhibited lower abundance of viral transcripts (CTL^+Gag^+ 7% versus CTL^-Gag^+ 4%, $p<0.0001$; Fig1), and was associated with relative depletion of *gag* in comparison to *nef*-containing transcripts (*gag:nef* ratio CTL^+Gag^+ 1:31 versus CTL^-Gag^+ 1:11, $p<0.0001$). In the no-superinfection control, viral transcripts derived from autologous proviruses were detectable at 0.006%, significantly lower than all other JR-CSF^+ conditions ($p<0.0001$).

Conclusions: Our Results: uncover nuances in the interplay between viral transcriptional regulation and susceptibility to CTL where, even within a clearly defined Gag^+ population, both quantitative and qualitative profile differences in viral transcripts were associated with survival. A greater understanding of this interplay may allow for improved efforts to expose viral reservoirs to elimination by CTL.

PP 3.6

HIV proviruses with identical sequences arise from cell expansion and infection by a common ancestor virus

S. Patro[1], A. Niyongabo[1], S. Guo[2], X. Wu[2], E. Boritz[3], S. Deeks[4], F. Maldarelli[1], S. Hughes[1], J. Coffin[5], M. Kearney[1]

[1] HIV Dynamics and Replication Program, National Cancer Institute, Frederick, USA, [2] Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, USA, [3] Virus Persistence and Dynamic Section, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, USA, [4] Department of Medicine, University of California San Francisco, San Francisco, USA, [5] Department of Molecular Biology and Microbiology, Tufts University, Boston, USA

Background: In individuals on ART, HIV-1 proviruses with identical sequences can arise from the proliferation of single infected cells or from genetic bottlenecks leading to viral clones that spread prior to ART. To investigate the origins of proviruses with identical sequences in individuals on ART, we sequenced near full-length proviruses and determined their sites of integration.

Methods: PBMC were obtained from 5 donors on ART and analyzed by Multiple-Displacement Amplification (MDA) Single-Genome Sequencing in which DNA is diluted to a proviral endpoint, whole genome amplified, and used for both integration site analysis and full-length proviral sequencing. 15 sets of identical sub-genomic sequences (12 in P6-PR-RT, 3 in env) were examined to determine if identical proviral sequences i) had identical integration sites (cell clones), ii) had different integration sites (viral clones), or iii) resulted from some with identical integration sites and some with different sites (both cell and viral clones).

Results: Of the 12 sets of identical P6-PR-RT sequences, 3 contained only identical integration sites (4, 2, and 2 MDA wells), 7 contained only different integration sites (2-5 MDA wells each), and 2 contained a combination of identical (19 and 13 wells) and different integration sites (4 and 2 wells). From the 3 sets of identical env populations, one had only different integration sites (7 wells) and 2 contained a combination of identical (3 and 28 MDA wells) and different integration sites (1 and 3 wells). Within the populations of defective proviruses, near-full length sequence analyses showed that proviruses with identical sub-genomic sequences were often identical throughout the genome, except for non-overlapping deletions, making it possible to reconstruct the sequence of the shared viral ancestor.

Conclusions: The finding that proviruses with identical sequences can have different integration sites demonstrates that sub-genomic SGS is not sufficient to identify clones of infected cells and suggests that multiple infected cell clones can be established from the same viral ancestor. Such viral clones can arise from the transmission bottleneck, escape from immune pressure, or selection for drug resistant virus.

PP 3.7

Diversity of the replication-competent HIV reservoir in treated patients

F. Mammano[1,2], A. Nicolas[1,2], J. Migraine[1], J. Dutrieux[1], M. Salmona[1,3], A. Tauzin[1,2], A. Hachiya[4], J.M. Molina[3], F. Clavel[3], A. Hance[1]

[1] Inserm U941, Paris, France, [2] Inserm U1124, Paris, France, [3] APHP, Hôpital Saint-Louis, Paris, France, [4] National Hospital Organization Nagoya Medical Center, Nagoya, Japan

Background: Viral genomes integrated in resting CD4+ T-lymphocytes are considered to be a major source of viruses reemerging after discontinuation of successful treatment. Replication competent proviral genomes represent a minority of the total proviral DNA. We evaluated the impact of treatment duration and the time between infection and effective treatment on the diversity of replication-competent viruses based on genome-wide sequences.

Methods: Resting CD4+ T-lymphocytes were isolate from 40 ml of peripheral blood from 8 successfully treated patients. Cells were stimulated and cultured under limiting dilution conditions with activated human CD4+ T-lymphocytes, to allow clonal virus outgrowth. Viral replication was monitored by p24 quantification, and between 4 and 14 (mean 8) individual viral isolates per patient were collected after short-term culture. The near full-length genomes of individual isolates were sequenced, and their single-cycle infectivity was compared.

Results: In 5 of the 8 patients, genotypically identical virus isolates were observed in independent wells, suggesting clonal expansion of infected cells in patients. Clonal viruses represented between 25 and 60% of the isolates (mean 48%). We also observed isolates sharing large identical portion of their genomes but displaying differences elsewhere, raising caution on the extrapolation of clonality from partial genome sequencing. Analyses of the mean pairwise distance

(MPD) of unique sequences for each patient showed that env and nef were the most variable genes. The MPD observed in different patients correlated with the time before treatment initiation (r=0.96, p=0.003), suggesting that the complexity of the replication-competent reservoir mirrors that present at treatment initiation. No correlation was observed between MPD and the duration of successful treatment (mean 8 years, range 2-15). Consistent with genotypic data, a broader range of infectivity (up to 100-fold) was observed among isolates from patients with delayed treatment initiation than in those treated early.

Conclusions: This work unveiled differences in the genotypic and phenotypic properties of reservoir viruses from treated patients, and suggests that delaying treatment Results: in increased diversity. These findings are relevant for strategies aiming the reduction of the reservoir.

Conflict of interest: JMM is a member of the advisory boards for Gilead, ViiV Healthcare, and Merck, and recipient of a grant from Gilead.

PP 3.8

Peripheral blood SIV/HIV originates from infected cells in tissues

M. Betts[1], L. Kuri Cervantes[1], M.B. Pampena[1], S. Samer[2], D. Khoury[3], I. Frank[1], M. Paiardini[2], M. Davenport[3], K. Bar[1], R. Veazey[4]

[1] University of Pennsylvania, Philadelphia, USA, [2] Emory University, Atlanta, USA, [3] Kirby Institute, Sydney, Australia, [4] Tulane National Primate Research Center, Convington, USA

Background: HIV and SIV infected CD4 T-cells localize primarily to lymphoid and mucosal tissues. While largely assumed, it remains to be established if peripheral blood (PB) viremia originates from these tissues, or from infected cells directly within the vasculature. We assessed in rhesus macaques (RM) and humans the potential contribution of tissue-based virus production to plasma viremia (pVL).

Methods: RM were infected with barcoded SIVmac239. Four animals (ART-) were treated with the lymphocyte migration inhibitor FTY720 daily from day 7 or 28 until day 90. Separately, fourteen animals started ART for six months and then underwent ART interruption (ART-TI). Of these, seven received FTY720 during AR-TI. PB and lymphoid tissue (LT) samples were collected for cell and virus quantification. We collected PB and thoracic duct lymph (TDL) from 11 HIV+ and assessed VL in each compartment. *Env* sequencing of plasma and TDL virus was used to characterize phylogeny.

Results: In the FTY720-treated RM we observed near complete redistribution of circulating CD4 T-cells into tissues within seven days of FTY720 treatment. Despite the absence of peripheral CD4 T-cells, all ART- animals had peak and set point pVL similar to historical controls. Likewise, in RM undergoing AR-TI, pVL rebound was equivalent in both FTY720-treated and untreated animals.

Barcode sequencing of cell-associated virus from LT and plasma virus during FTY720 treatment revealed substantial overlap in the dominant virus populations replicating in LT and circulating in plasma. These Results: suggest that the circulating plasma virus originated from tissues.

We next assessed paired TDL and plasma from HIV+ donors. HIV RNA copies were higher in TDL vs PB, and virus populations were phylogenetically indistinguishable between the compartments. Based upon differential VLs, viral clearance rate, plasma volume, and lymph output we calculated that ~50% of plasma virus originates from thoracic duct output, in some individuals reaching a 100% contribution.

Conclusions: Our Results: indicate that HIV infected cells within LT and non-LT, rather than the vasculature, are the major source of peripheral viremia. A large proportion of this viremia is maintained through thoracic duct lymphatic efflux, indicating that virus released from infected cells in tissues travels through lymphatics into blood.

PP 3.9

Ultradeep analysis of pretherapy HIV predicts large and genetically complex reservoirs during antiretroviral therapy

K. Huik[1], J. Hattori[1], V. Boltz[1], J. Rausch[1], W. Shao[2], M. Kearney[1], J. Coffin[3], F. Maldarelli[1]

[1] HIV Dynamics and Replication Program, NCI, NIH, Frederick, USA, [2] Leidos Biomedical Research, Frederick, USA, [3] Tufts University, Boston, USA

Background: Measuring the genetic characteristics of HIV populations is essential to understanding the formation of HIV reservoirs that persist during antiretroviral therapy (ART). Analysis of plasma HIV by new next generation sequencing (NGS) approaches using primer-ID [ultrasensitive single genome sequencing (uSGS)] and advanced bioinformatic analyses (Boltz et al. 2016), yields large HIV sequence datasets with the same, low PCR error and recombination rate as standard SGS. We used uSGS (i) to determine HIV replicating population size and in vivo recombination rate, and (ii) to modify uSGS to characterize cell associated (CA) HIV RNA and DNA derived from peripheral blood lymphocytes (PBLs).

Methods: Chronically-infected, ART naïve individuals (N=6) were enrolled in studies of HIV infection at the NIH in 2000-2002. uSGS of HIV RT [HXB2nt 2704-2943 and 3046-3253] yielded c. 400-nt sequences. Replicating population sizes were estimated as previously described (Maldarelli et al. 2013), linkage disequilibrium measurements were calculated using DNASP, and recombination rates were calculated by measuring the rate at which linked alleles became unlinked. To modify uSGS to proviral DNA, genomic DNA extracted from PBL was sheared (average of 10 kb) and linear PCR was used to add the primer-IDs prior to the uSGS procedure.

Results: Longitudinal plasma samples were obtained from chronically-infected ART naïve subjects (median CD4=498 cells/μl, viral RNA=4.3 log_{10} copies/ml). A total of 17,172 (median 1,252/patient, range 54-3,165) plasma RNA sequences were obtained by uSGS from 2 timepoints each. Maximum replicating population sizes exceeded 10^7/person. Viral populations were highly polymorphic, but nearly all polymorphisms were in linkage equilibrium. With a single exception, all linked loci (3-12 loci/donor) became unlinked over short periods (30-413 generations). The measured recombination rate (range 0.004-0.07) is similar to previous estimates (Batorsky et al. 2011). Analysis of uSGS from the PBL of one donor revealed HIV was readily recovered with 742 DNA sequences and 946 RNA sequences.

Conclusions: Prior to ART, HIV populations are large (>10^6-10^7/person) and composed of variants that are virtually all the product of recent recombination events. uSGS predicts viruses rebounding from reservoirs will be diverse and likely to have evidence of prior recombination events.

PP 3.10

In vivo analysis of HIV from an occupational exposure to laboratory adapted HIV-IIIB with 20-year follow-up: implications for reservoir formation

C. Lange[1], N. Lindo[2], R. Little[2], T. Uldrick[3], S. Hill[1], J. Bell[2], K. Lurain[2], R. Ramaswami[2], R. Yarchoan[2], F. Maldarelli[2]

[1] National Cancer Institute, Frederick, USA, [2] National Cancer Institute, Bethesda, USA, [3] Fred Hutch Cancer Research Center, Seattle, USA

Background: HIV infection via laboratory exposure is rare, and the long term effects on HIV reservoirs have not been investigated. Here we characterized an individual infected with CXCR4 tropic HIV-IIIB acquired via occupational exposure in 1985.

Methods: Clinical records from NIH Clinical Center were reviewed, and available stored plasma from 1997-2012 were retrieved for analysis. HIV RNA was extracted from plasma and subjected to single genome sequencing (SGS) of HIV *pro-pol;* SGS (N=106 sequences) were

aligned and subjected to phylogenetic and population genetic analyses; Drug resistance mutations were identified using Stanford Database.

Results: The study participant is a white male HIV infected with IIIB laboratory strain of HIV, which has a stop codon in the second coding exon of *tat*, in 1985 via occupational exposure. He had no symptoms of acute HIV infection, but seroconverted and experienced progressive CD4 lymphopenia. He participated in a therapeutic gp160 vaccine study and CD4 cell numbers declined 156 cells/µl by 1996. He initiated antiretroviral (ARV) therapy with AZT+3TC and had increases in CD4 >350 cells/µl. When viral RNA testing was available, HIV RNA levels were initially <500 c/ml (limit of detection) but rebounded to 800 c/ml after 1 y of AZT+3TC with the emergence of M184V. Subsequent therapy was characterized by low level viremia and compromised by severe ARV adverse effects; he underwent intermittent ARV therapy from 2002-2008, and CD4 declined to 200 cells/µl. In 2010 he initiated Tenofovir DF+FTC+Efavirenz and had HIV viremia suppressed until the present. Analysis of pre-therapy plasma derived HIV RNA 11 years after infection revealed HIV remained highly related to IIIB with only 4-11 nt changes from IIIB among all pretherapy variants. Populations had limited genetic diversity (average pairwise difference 0.5% after 11 years of untreated infection). Genetic diversity accumulated slowly from 1997-2010 but remained <1 % even after >20 years of infection.

Conclusions: Laboratory adapted, X4 tropic HIV-IIIB causes AIDS in vivo. Viral populations remained highly similar to the founder virus. Relatively slow change in virus diversity over time indicates the HIV reservoir was formed from a large and uniform population that persisted for years.

PP 3.11

Post-ATI viremia in a hyperacute subject comprises macrophage-tropic viral variants

V. Machado[1], M. Sharkey[1], T. Cordeiro[1], L. Barrios[1], T. Henrich[2], M. Stevenson[1]

[1] University of Miami, Miami, USA, [2] University of California San Francisco, San Francisco, USA

Background: HIV-1 persists in cellular reservoirs that cannot be eliminated by current antiretroviral therapy (ART). Early HIV-1 infection involves CCR5-using, T-cell tropic viruses that can establish persistent viral reservoirs rapidly following initial infection. Thus, understanding the nature of the cellular reservoirs that are established after acute infection is crucial for the development of preventative strategies.

Methods: We characterized the cellular tropism of multiple HIV-1 envelopes from post-ATI plasma isolated from a hyperacute individual who initiated ART 12 days post-HIV-1 exposure. Recombinant viruses were prepared from 32 full-length envelopes generated by Single Genome Amplification (SGA). Recombinant viruses were then assessed for their ability to fuse with, and replicate within CD4+ T cells and primary macrophages.

Results: HIV-1 envelopes isolated from this hyperacute subject showed an increased capacity to fuse with macrophages. Infectious capacity for CD4+ T cells did not vary significantly among the envelopes tested. Of 32 envelopes examined, 19 were clonal and T-cell tropic. A single envelope was found to fuse and replicate efficiently within macrophages. Macrophage tropism was governed by a single amino acid substitution (L125F) on a CD4-binding site upstream of the envelope V1 region. Mutagenesis revealed that this L125F mutation was necessary and sufficient for macrophage tropism. Previous studies suggest that this mutation was present in HIV-1 strains that are preferentially transmitted in HIV-discordant couples (Redd et al., 2012), and in viruses resistant to neutralization by broadly neutralizing antibody VRC01 (Lynch et al., 2015).

Conclusions: Since this subject was placed on ART within 12 days of infection, there was limited opportunity for continued evolution of the viral population that was initially transmitted. Therefore, our findings suggest that a macrophage-tropic, CCR5-using HIV-1 may have been transmitted during HIV-1 infection, and that this variant persisted during suppressive ART.

PP 3.12

Long-term persistence of HIV-infected cell clones in early treated children

M. Bale[1], M.G. Katusiime[2], D. Wells[3], X. Wu[3], J. Coffin[4], M. Cotton[5], S. Hughes[1], J. Mellors[6], G. Van Zyl[2], M. Kearney[2]

[1] HIV Dynamics and Replication Program, CCR, National Cancer Institute, Frederick, USA, [2] Division of Medical Virology, Stellanbosch University, Cape Town, South Africa, [3] Leidos Biomedical Research Inc., Frederick, USA, [4] Department of Molecular Biology and Microbiology, Tufts University, Boston, USA, [5] Department of Pediatrics and Child Health, Tygerberg Children's Hospital and Family Clinical Research Unit, Cape Town, South Africa, [6] Department of Medicine, University of Pittsburgh, Pittsburgh, USA

Background: HIV-1 integration site analysis performed on PBMC collected from adults showed that infected T cell clones arise in acute infection and persist for many years on antiretroviral therapy (ART). However, little is known about the dynamics of infected T cell clones in vertically-infected children who start ART shortly after birth.

Methods: Samples were obtained from 12 perinatally-infected children who were treated early and whose viremia was fully suppressed on ART for the duration of treatment. We obtained HIV-1 integration sites from samples taken prior to ART initiation and after 6-9 years on ART. We compared the in vivo integration site distributions to the distributions in ex vivo infected PBMC to look for evidence of selection for proviruses integrated in specific genes or regions.

Results: We obtained 8,841 integration sites prior to ART and 1,860 sites on ART from 11 of the 12 children. We found that, similar to what has been reported in adults, clones of infected cells arose early (clones were detected in 10 of the 11 children pre-ART) and persisted for up to 9 years on ART (clones were detected in all children on ART with 8 children having integration site matches between pre-ART and on-ART samples). By comparing integration sites obtained from the on-ART samples to the sites obtained from PBMC infected ex-vivo, we show that there was selection for cells with proviruses integrated in BACH2 and STAT5B.

Conclusions: Despite marked differences in T cell dynamics between children and adults, integration sites show that the landscape and persistence of HIV-1 infected cell clones are similar. In children, clonal expansion of infected cells occurred shortly after birth, the clones persisted for many years on ART, and there was selection for proviruses integrated in some of the same genes as found in HIV-1 infected adults.

Conflict of interest: John Mellors is a consult to and has received grants from Gilead Sciences and owns shares of Co-crystal Pharmaceuticals, Inc. The remaining authors have declared that no conflict of interest exist.

PP 3.13

CXCR4-usage HIV-1 strains isolated from blood and cerebrospinal fluid in subjects on suppressive antiretroviral therapy

A. Nath[1], G. Li[1], L. Henderson[1], B. Smith[1], L. Reoma[1], X. Jiao[2], U. Santamaria[1], H. Imamichi[2], C. Lane[3]

[1] NIH/NINDS, Bethesda, USA, [2] NIH/NIAID, Frederick, USA, [3] NIH/NIAID, Bethesda, USA

Background: Studies show that the brain is an important HIV reservoir and CSF viral escape is broadly investigated as a predictor of the CNS reservoir among HIV+ patients on cART. CCR5-usage viruses are predominantly found in the CNS. Here, we report Results: of HIV outgrowth from both blood and CSF of patients with suppressive cART.

Methods: 100-150 ml of CSF via lumbar drain and 10 ml of blood were collected from 9 subjects with suppressive cART. CSF cells and PBMCs were cocultured with CD8-depeleted, PHA-stimulated donor PBMCs. HIV isolates were tested for tropism and infectivity in

MDMs or human astrocytes. Both PacBio and MiSeq were respectively employed for sequencing full-length gp120 or V3 loop of HIV isolates.

Results: Total 9 patients were selected for HIV outgrowth from the cells of lumbar-drained CSF and PBMCs; 7 of them had normal cognitive performance and two had HAND. Except for one case, HIV-1 RNA was not detected in blood and CSF. We isolated 7 HIV-1 strains, one from CSF cells and 6 from PBMCs in 5 patients including two with HAND. 6 of 7 viruses were characterized for tropism, 5 were X4-tropic and one was R5-tropic. Interestingly, the patient with one isolate from CSF and 2 isolates from blood at two time-points had normal cognition and all of 3 viruses were X4-tropic. These 3 viruses and another one were further sequenced for full-length gp120 or V3 loop. Analysis of phylogenetic trees showed that CSF viral quasispecies were compartmentalized and could seed the periphery. Furthermore, three viruses from the single patient productively infected astrocytes via a transwell culture system where HIV-infected JKT cells were loaded on top chamber and only HIV particles could go through its membrane and reach astrocytes in bottom wells. This is consistent with prior observations that immature HIV particles released from HIV-infected lymphocytes were able to directly bind to CXCR4 on astrocytes leading to the infection in absence of CD4.

Conclusions: X4-tropic viruses may predominate in the patients with long-term cART-suppressive therapy. X4 virus can be compartmentalized in the CSF and over a period of time seed the periphery.

PP 3.14

Marked variation in the susceptibility of HIV-1 to type 1 interferon inhibition during early, late and rebound infection

M. Gondim[1], S. Sherrill-Mix[1], M. Saag[2], M. Nussenzweig[3], J. Silicano[4], P. Sharp[5], P. Borrow[6], L. Montaner[7], K. Bar[1], B. Hahn[1]

[1] Department of Medicine, University of Pennsylvania, Philadelphia, USA, [2] Department of Medicine, University of Alabama at Birmingham, Birmingham, USA, [3] Laboratory of Molecular Immunology, Rockefeller University, New York, USA, [4] Department of Medicine, Johns Hopkins University, Baltimore, USA, [5] Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK, [6] Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK, [7] Vaccine and Immunotherapy Center, Wistar Institute, Philadelphia, USA

Background: Type 1 interferons (IFNs) mediate antiviral control through a number of immune-regulatory mechanisms, including the expression of interferon-stimulated genes. We have previously reported that virus resistance to type 1 IFNs is a key determinant of HIV-1 transmission fitness.

Methods: To examine the kinetics of IFN-mediated control during HIV-1 infection, we generated 470 plasma and peripheral blood mononuclear cell-derived viral isolates from 10 longitudinally studied subjects, 14 subjects on suppressive antiretroviral therapy (ART), and 13 subjects undergoing analytic treatment interruption (ATI). Interferon dose-response experiments were performed on all virus isolates to determine the concentration of interferon that decreased replication by 50% (IC50).

Results: Determining the IFN concentration that reduced viral replication by 50% (IC50), we found that isolates generated during acute infection were highly IFNa2 and IFNb resistant (mean: 0.63 and 9.2 pg/ml, respectively). This resistance decreased in most subjects in the first two years post-infection (mean: 131- and 1,690-fold, respectively), was partially reacquired concomitant with disease progression (mean: 10.9- to 152-fold, respectively), and remained elevated in subjects with an accelerated disease course. Isolates generated by viral outgrowth during suppressive ART were only moderately IFNa2 and IFNb resistant (mean: 0.064 and 0.84 pg/ml, respectively), resembling plasma viruses sampled immediately prior to ART. In contrast, viruses isolated from post-ATI rebound plasma were highly IFNa2 and IFNb resistant (mean: 2.1 and 64.9 pg/ml, respectively), exceeding values of acute infection isolates.

Conclusions: These Results: indicate a highly dynamic role of type 1 IFNs throughout the course of HIV-1 infection, including viral rebound. Importantly, they suggest that as in acute infection, type 1 IFNs place pressure on the rebounding virus pool, limiting the viruses that can successfully reactivate from latency. The heightened IFN resistance of rebound viruses may provide an avenue to identify their cell and tissue origins.

PP 3.15

Single-cell multiplexed RNA flow-FISH analysis of primary human samples reveals distinct VR reactivation profiles among LRA classes and curtailed VR transcriptional and translational reactivation patterns by HDAC inhibitors

D. Kaufmann[1], G. Sannier[1], M. Dubé[1], N. Brassard[1], G.G. Delgado[1], A. Baxter[1], J.P. Routy[2], N. Chomont[1]

[1] Research Centre of the Centre Hospitalier de l'Université de Montréal, Université de Montréal, Montreal, Canada, [2] Chronic Viral Illnesses Service and Division of Hematology, McGill University Health Centre, Montreal, Canada

Background: 'shock and kill' cure strategies rely on efficient Latency Reversal Agents (LRAs) capable of reactivating viral reservoir (VR) cells and making them recognizable by the immune system. While a better understanding of the high diversity of VR generated in vivois key to achieve cure, its study at the single-cell level in primary samples is challenging.

Methods: We developed a multiplexed RNA flow cytometric fluorescent in situ hybridization (RNA flow-FISH) assay for simultaneous detection of three viral RNAs, p24 protein and cellular markers. We did an observational study on PBMC from 7 ART-suppressed people (ART) and 5 uninfected controls. We examined VR transcription and translation induced by PMA/ionomycin (P/I), HDAC inhibitors (Panobinostat, Vorinostat; HDACi), and PKC agonists (bryostatin, PEP005; PKCa). Data were analyzed by FlowJo and Prism.

Results: This novel assay allows sensitive and specific single-cell analysis of VR upon LRA stimulation. In ART, median vRNA+VR frequencies/106CD4 induced by LRAs were 51 for P/I, 22 for PEP005 and 15 for Panobinostat. Upon LRA stimulation, only a small fraction of the vRNA+ VR expressed p24 protein (median 8% for P/I, 18% for PEP005 and 5% for Panobinostat). While HIV gagand neftranscripts were highly co-expressed in vRNA+p24+ VR, their patterns were heterogeneous in vRNA+p24- cells. Mean vRNA+VR subset frequencies induced by PEP005 were: gag+nef+32%; gag+nef- 31%; gag-nef+ 37%. In contrast, HDACi induced an abortive pattern of VR transcription: for Panobinostat, mean frequencies were gag+nef+ 13%; gag+nef- 71%; gag-nef14%. These profiles were consistent within LRA classes.

Conclusions: We identified distinct single-cell patterns of VR transcription and translation induced by LRAs, this in primary clinical samples. In ART, dissociated expression of structural and regulatory genes was frequent, and differences observed between LRA classes. Irrespective of the LRA tested, only a small minority of the vRNA+ VR cells were triggered to produce p24 protein, an immunodominant target for CD8 T cell responses. HDACi preferentially induced a gag+nef- p24- population, suggesting blocks in progression of transcription. These findings have implications for both 'shock' and 'kill' interventions. The multiplexed RNA Flow-FISH approach can be relevant for monitoring of clinical cure trials.

PP 3.16

HIV-1 sequence compartmentalisation and evolution in CNS and immune tissue

M. Gonzalez-Perez[1], R. Rose[2], D.J. Nolan[2], S.L. Lamers[2], P.R. Clapham[1], K. Luzuriaga[1]

[1] University of Massachusetts Medical School, Worcester, USA, [2] BioInfoExperts LLC, Thibodaux, USA

Background: HIV-1 can be detected in the CNS at all stages of infection; however, the timing of infection and evolutionary pathways have not been well-delineated. Here, we investigated the genetic patterns of the virus from CNS and lymphoid tissues from 7 patients with and without Neuro-AIDS.

Methods: DNA was extracted from 12 post-mortem tissues (frontal lobe, cerebellum and spleen) and HIV-1 env genes were sequenced using PacBio SMRT technology. Sequences were filtered for quality and aligned using MAFFT and AliView, resulting in 'Optimized Alignments' (range 12,650 – 44,368 sequences/tissue). The number of glycosylation sites, length, charge and predicted co-receptor usage were evaluated for env variable regions (V1-V5).

For each tissue, high-quality consensus sequence (HQCS) alignments were produced by removing the variable regions and grouping sequences with 99% genetic similarity. HQCS10 alignments were then generated by including only variants that represented >10 reads. HQCS10 sequences from all patients/tissues were combined, and used to infer a maximum likelihood ML tree. Genetic clusters were identified using a hierarchical strategy based on genetic distance thresholds at 1%-5%.

Results: Patients were distinctly separated into clades on the HQCS10 ML tree, and in each patient with multiple tissues, brain sequences formed a distinct and separate clade with respect to spleen sequences. Branch lengths were highly variable among patients.

To quantify variation and structure among patients' tissues, sequences were clustered using a range of genetic distance thresholds. At the lowest threshold (1%), tissue sequences from all but one patient were separated into distinct clusters. As genetic distance increased, the number of clusters per patient decreased and tissue sequences merged together. A patient without Neuro-AIDS showed the highest diversity between brain and spleen. Variable region analysis of the Optimized Alignments showed similar variability within and among patients. Taken together, these patterns are consistent with continued viral evolution in the brain reservoir.

Conclusions: While minor correlations were found between genetic diversity, disease, length of infection and drug use, it was clear that brain and spleen tissues are compartmentalized in patients with long-term infection, with and without neuro-AIDS.

PP 3.17

Intact and replication-competent reservoir virus populations differ from each other and rebound plasma viruses

K. Bar, F. Mampe, M.A. Monroy, E. Lindemuth, L. Kuri Cervantes, F. Bibollet-Ruche, B. Hahn, D.B. Salantes

University of Pennsylvania, Philadelphia, USA

Background: Accurate methods to identify the persistent reservoir are a priority. Here, we compare reservoir sampling methods and rebound viruses in 3 ART-suppressed individuals undergoing ATI.

Methods: In 3 participants, a total of 257 single genome sequencing (SGS)-derived env and 99 near-full length (NFL) sequences of proviral DNA from CD4 T cells, env sequences from 107 limiting-dilution VOA cultures, and 196 env sequences from T_{naive}, T_{EM}, T_{CM}, and T_{TM} CD4 T cells were generated and compared with 70 env sequences from the first detectable rebound viremia post-ATI.

Results: In participant A, env sequencing of proviral DNA identified multiple (n=13) groups of identical sequences. These putative clones represented 47% of env sequences. NFL sequencing revealed that just 3 of the 13 clones had intact genomes. Two intact clones comprised the majority of NFL sequences (28% and 55%, respectively). Sequences of participant A's VOA cultures identified both clones as replication competent, but with substantially different frequencies (95% and 2%, respectively) than in proviral DNA.

In participants B and C, multiple putative clones were identified by env sequencing; each participant had one expanded clone (representing 44% and 75% of sequences, respectively). NFL sequencing confirmed intact genomes in both clones, but neither were recovered in VOAs.

In all participants, T cell subset sequencing revealed that expanded proviral DNA clones not recovered by VOAs were from TEM cells. In all participants, NFL and VOA sequences did not overlap with rebound viruses. VOAs performed with a variety of conditions, including a range of cytokine treatments and activation methods, failed to increase detection of intact proviral DNA clones or rebound viruses.

Conclusions: Sampling the latent reservoir is challenging and current methods have distinct biases. Expanded T_{EM} clones with intact genomes were commonly detected via sequencing, but undersampled by VOAs. Modifications of VOA conditions did not increase yields of expanded, intact T_{EM} clones, nor improve detection of rebound viruses. Enhanced methods to sample reservoir viruses capable of rebound, including methods to sample rare, non-CD4 T cell, or tissue-based populations are needed.

PP 3.18

Longitudinal sequencing reveals multiphasic decay of HIV reservoir

L. Cannon, M. Pinzone, E. Venanzi-Rullo, U. O'Doherty

University of Pennsylvania, Philadelphia, USA

Background: The behaviour of the reservoir on ART is poorly understood and insight into how it changes over time could provide a better understanding of the mechanisms that lead to HIV persistence and potentially lead to a cure strategy. We seek to provide further clarification of the dynamics of the reservoir via longitudinal sequencing of HIV proviruses.

Methods: We sequenced 2000+ provirus from longitudinal samples, from chronically infected HIV patients, over a period of approximately 10 years and obtained ~100 near full length provirus at each time point by utilizing a nested PCR technique at limiting dilution. Total HIV DNA was measured by RU5 qPCR. Intact proviruses were defined as those that contained a complete psi packaging site, 9 intact ORFs (including known functional variants), RRE, TAR, undamaged stem loops, and the presence of critical splice donor and acceptors sites. We estimated the level of replication competent provirus at each time point. A mixed-effect modeling approach revealed multiphasic decay of the reservoir.

Results: Both intact and defective HIV DNA appear to follow multiple distinct phases of decay over the sampling period. The first phase of decay is characterized by a quick decline over the first two months on ART. The second phase of decay is far more gradual and transpires over seven years following ART initiation. The last phase of decay, which dominates after approximately seven years of ART, is stable with a decay rate not statistically different from zero. Analysis of proviral sequences during each of these phases reveals consistent changes in deletion patterns and clonal prevalence.

Conclusions: We found the HIV reservoir decay to be multi-phasic. These phases likely correspond to dynamics of distinct subpopulations of infected T cells. It is clear that post integration selection both positive and negative are critical to the formation of a truly stable reservoir. This work revealed disparate selection pressures and significant clonal expansion, which may indicate underlying persistence mechanisms.

Conflict of interest: This is an updated version of submission (00149 - Longitudinal Sequencing Reveals Multiphasic Decay of HIV Reservoir).

Session 4: Immunology of HIV persistence

OP 4.2

Characterising 'exceptional' control among HIV elite controllers

M. Peluso[1], P. Burbelo[2], S. Kumar[1], S. Munter[1], R. Hoh[1], S. Lee[1], P. Hunt[1], R. Rutishauser[1], T. Henrich[1], S. Deeks[1]

[1] *University of California, San Francisco, San Francisco, USA,* [2] *National Institutes of Health, Bethesda, USA*

Background: Studies of 'elite controllers' (ECs) might lead to novel approaches for HIV cure. We characterized the clinical, immunologic and virologic characteristics of ECs with very low reservoirs ('exceptional controllers'). Such individuals may prove to be models for a functional cure.

Methods: We applied a clinical case definition to identify ECs within the SCOPE cohort. A related ART-treated cohort (n=80) was used for comparison. We measured CD4 T cell-associated (CA)-HIV DNA and CA-RNA using PCR from median 9M PBMCs, HIV-specific antibody responses using luciferase immunoprecipitation systems (LIPS), and T cell responses using flow cytometry. We stratified the sample by reservoir size and compared clinical outcomes, antibody response, and T cell immunophenotypes. Exceptional controllers were defined as ECs with no detectable HIV DNA. Clinical progression was defined as loss of virus control or CD4 decline requiring ART.

Results: 96 individuals met our case definition. Median CA-DNA and CA-RNA was 1.5 (0-7.6) and 99 (4.8-317) copies/10∧6 cells, respectively. These levels were significantly lower than those on ART (CA-DNA 10.8 and CA-RNA 2138 copies/10∧6 cells, p<0.001 for both). CA-DNA levels highly correlated with CA-RNA levels (0.74, p<0.001). CA-DNA levels were associated with antibody levels targeting matrix (r=0.30, p=0.008), integrase (r=0.26, p=0.03), and protease (r=0.27, p=0.02), but not envelope, or measures of T cell activation. 22 (23%) met our virologic definition of exceptional control. Exceptional controllers were more likely to have a protective HLA allele (B27 or 57; p=0.002) and less likely to progress clinically (18% vs 49%, p=0.02). Compared with the rest of the EC cohort, exceptional controllers had lower antibody levels to matrix (p=0.007), integrase (p=0.007), and protease (p=0.02), but comparable levels of T cell activation. In a logistic regression model, exceptional control was associated with presence of protective HLA alleles (6.8 fold effect, p=0.002).

Conclusions: We identified a subset of controllers with very low HIV DNA and RNA levels, low HIV antibody levels, and lower risk of clinical progression. These individuals are enriched for certain HLA alleles, arguing that CD8+ T cell responses mediate control. Such individuals may not need ART and might prove to be a model for a 'functional cure.'

OP 4.3

Single-cell TCR sequencing reveals that clonally expanded cells highly contribute to the inducible HIV reservoir during ART

P. Gantner[1], A. Pagliuzza[2], M. Pardons[1], M. Ramgopal[3], J.P. Routy[3], R. Fromentin[2], N. Chomont[1]

[1] *Université de Montréal, Montreal, Canada,* [2] *CRCHUM, Montreal, Canada,* [3] *Midway Immunology and Research Center, Fort Pierce, USA*

Background: Clonal expansions occur in the persistent HIV reservoir as demonstrated by the duplication of HIV genes and/or integration sites reported in several studies. However, these approaches do not permit to analyze the phenotype of these expanded clones of infected cells nor the inducibility of the proviruses. We took advantage of the uniqueness of the T-cell receptor (TCR) within a given T-cell clone to unravel the phenotype and dynamics of the inducible HIV reservoir at the single cell level.

Methods: Longitudinal blood samples from 8 individuals on suppressive ART were analyzed. Clonotype characterization of HIV-infected cells was determined by combining index single-cell sorting of HIV-infected cells by HIV-Flow (which allows recording the memory phenotype of individual p24+ cells) with multiplex PCR of the V-J junction of the TCRβ chain (including the CDR3 region) followed by sequencing. A representative subset of p24- cells was analyzed to determine TCR diversity in the uninfected population of cells.

Results: We obtained the TCRβ sequences from 538 p24+ and 346 p24- single-sorted cells. There was no bias in the selection of V and J segments in p24+ cells when compared to p24- cells. Expanded infected clonotypes sharing the same TCR sequence were detected in 7/8 individuals and accounted for the majority of reservoir cells (median 89%, range, 77-100). Expanded clonotypes were maintained over time on ART in 5 participants, but their proportions greatly varied. Interestingly, one of the expanded p24+ clonotypehad been previously described as reactive against the gut microbiota. Expanded reservoir cells were systematically detected among different memory subsets and overrepresented in the most differentiated populations (i.e. transitional and effector memory).

Conclusions: We developed a method to obtain TCR sequences from single HIV-infected cells in individuals on ART. Using this approach, we demonstrate that: (1) clonal expansion highly contributes to the persistence of the translation-competent HIV reservoir, (2) clonally-expanded cells display multiple memory phenotypes, and (3) the dynamic of the repertoire of the HIV reservoir varies between individuals. Our Results: suggest that antigen stimulation is a major driver of the HIV reservoir dynamics during ART.

OP 4.4

Single-cell phenotyping of HIV-infected expanded clones in ART-suppressed individuals

C. Dufour[1], M. Pardons[1], R. Fromentin[1], M. Massanella[1], S. Palmer[2], S. Deeks[3], B. Murrell[4], J.P. Routy[5], N. Chomont[1]

[1] *Centre de Recherche du CHUM and Department of Microbiology, Infectiology and Immunology, Université de Montréal, Montreal, Canada,* [2] *Centre for Virus Research, Westmead Institute of Medical Research, University of Sydney, Sydney, Australia,* [3] *Department of Medicine, University of California San Francisco, San Francisco, California, USA,* [4] *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden,* [5] *Division of Hematology and Chronic Viral Illness Service, McGill University Heath Centre, Montreal, Canada*

Background: Eradicating HIV remains an ongoing challenge due to the presence of persistent viral reservoirs. Several studies have attempted to identify the phenotype of the cells harboring persistent HIV but have been hampered by the limited throughput offered by flow cytometry cell sorting and the challenge of amplifying full length genomes from bulk sorted populations. Here, we developed a novel single cell approach to identify biomarkers of cells harboring near full length HIV genomes in individuals on ART.

Methods: Large number of *in vitro* stimulated CD4+ T cells from 4 ART-treated participants were single cell sorted based on p24 expression using HIV-Flow. Levels of expressions of CD45RA, CCR7, PD-1, TIGIT, ICOS, HLA-DR, integrins α4 and β1 were simultaneously measured. Using a modified version of the FLIPS assay, individual proviruses from single sorted cells were amplified by nested-PCR. Near full-length viral genomes were sequenced by PacBio to establish their integrity and clonality, which were further associated with the expression levels of each cell-surface marker.

Results: We obtained 51 proviral sequences from single sorted p24+ cells. Among the 41 sequences that were >8.5 kb long, all but one were defective. We identified 6 clusters of identical sequences, indicating clonal viral expansions within the pool of p24+ cells. The vast majority of individual cells from these expanded clones displayed a CD45RA-CCR7- α4+ phenotype. However, the expression levels of β1, HLA-DR, ICOS, PD-1 and TIGIT were highly variable between cells sharing the same HIV genome, indicating a large diversity in the phenotype of clonally expanded infected cells.

Conclusions: We efficiently combined index single cell sorting of p24-expressing cells with near full-length HIV genome sequencing to characterize individual infected cells from ART-treated individuals. Our analysis reveals the diversity in the phenotype of cells harboring identical proviral sequence and suggests that clonal expansions of HIV genomes contributes to the diversification of the pool of HIV reservoir cells.

OP 4.5

The IciStem consortium: T-cell immunology in HIV-1 infected individuals after allogeneic stem cell transplantation

J. Eberhard[1], M. Angin[2], C. Passaes[2], M. Salgado[3], J.L. Díez Martín[4], M. Nijhuis[5], A. Wensing[5], J. Martinez-Picado[3], J. Schulze Zur Wiesch[1], A. Sáez-Cirión[2]

[1] Department of Medicine, Infectious Diseases Unit, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2] Institut Pasteur, HIV, Inflammation and Persistence, Paris, France, [3] AIDS Research Institute IrsiCaixa, Barcelona, Spain, [4] Hospital Universitario Gregorio Marañón, Instituto de Investigación Sanitarias Gregorio Marañón, Universidad Complutense, Madrid, Spain, [5] University Medical Center, Utrecht, the Netherlands

Background: So far the only medical intervention to cure HIV, has been hematopoietic allogeneic stem cell transplantation (allo-HSCT). While the size of the HIV reservoir sharply decreases after allo-HSCT, little is known about the immunological reconstitution and possible predictors of eradication or persistence of the HIV reservoir in the setting of allo-HSCT.

Methods: The IciStem project includes to date 45 HIV-infected subjects who received an allogenic transplant. PBMC samples were available from 16 patients (4/16 with CCR5D32/D32 graft). Phenotype and function of T-cell populations were characterized via flow cytometry. To estimate the breadth and quality of the HIV-specific T-cell responses, T-cells were stimulated with Gag, Pol and Nef peptide pools, and production of cytokines (IL-2, IFN-g, TNFa) as well as degranulation (CD107a) were measured via ICS and compared to responses against CMV-derived peptides.

Results: We found a slow, heterogeneous T-cell reconstitution with initial expansion of activated CD4+T-cells preceding the expansion of CD8+T-cells. While HIV-specific CD8+T-cell responses disappeared immediately after allo-HSCT, weak ex vivo responses were detectable several weeks after allo-HSCT, and could still be detected at the time of full T-cell chimerism, indicating that de novo priming occurred during the time of T-cell expansion. These HIV-specific T-cells had limited functionality and persisted years after allo-HSCT.

Conclusions: Immune reconstitution after allo-HSCT was slow and heterogeneous in the study participants. There was an initial phase of high T cell activation and exhaustion which renders a possibility of reinfection of engrafted cells. This was accompanied by the de novo priming of weak HIV-specific T cell responses with limited functionality. Our findings point out the importance of maintaining ART during the first months after HSCT.

OP 4.6

Dynamics of HIV-specific T cells on long-term ART differ by antigen recognised and by sex

Eva M. Stevenson[1], Adam R. Ward[1,2,3], Thomas R. Dilling[1], John K. Bui[1], John Mellors[4], Rajesh Gandhi[5], Deborah McMahon[4], Joseph Eron[6], Ronald Bosch[7], Christina Lalama[7], Joshua Cyktor[4], and Brad Jones[1,2], for the A5321 Team

[1] Division of Infectious Diseases, Weill Cornell Medicine, New York, NY USA, [2] Department of Microbiology, Immunology, and Tropical Medicine, George Washington University, Washington, DC USA, [3] PhD program in Epidemiology, George Washington University, Washington, DC USA, [4] University of Pittsburgh, Pittsburgh, PA, USA, [5] Massachusetts General Hospital, Boston, MA, USA, [6] University of North Carolina, Chapel Hill, NC, USA, [7] Harvard University, Boston, MA, USA

Background: T-cell responses to HIV decay in early ART, with a 39-week half-life. Previously, we showed a direct correlation between levels of cell-associated HIV DNA (CA-DNA) and magnitudes of HIV-specific T-cell responses targeting early gene products Nef/Tat/Rev in the ACTG A5321 cohort. Our results suggested that ongoing interactions with HIV-infected cells may shape HIV-specific T-cell responses on long-term ART; however, little is known about response dynamics.

Methods: We performed IFN-γ ELISPOT assays on 49 participants' PBMCs from on-ART timepoint 1 (study entry - median [IQR] years on ART 7 [4, 8]) to assay responses to HIV peptide pools Gag, Env, Pol, Nef/Tat/Rev, as well as CMVpp65. Here, we repeated with PBMCs from weeks 24 and 168 post-entry. We assessed relationships between these responses and virologic/immunologic and clinical data provided by the ACTG.

Results: HIV-specific T-cell responses were stable on durable ART, with magnitudes differing by gene product and by sex (Figure). Responses exhibited long median half-lives, also differing by sex: Gag 32.4yrs (F 75.1yrs, M 3.7yrs); Env 3.5yrs (F 2.5yrs, M 1.1yrs); Pol 12.1yrs (F no decay, M 6.1yrs); Nef/Tat/Rev 6.9yrs (F 5.3yrs, M 6.1yrs). F vs. M participants had higher magnitudes of responses longitudinally for all HIV gene products, but not for CMV, before and after controlling for pre-ART HIV RNA and CD4 count (all $p<0.05$). Higher levels of CA-DNA at study entry associated with lesser decay of Nef/Tat/Rev-specific responses between weeks 24 and 168 ($r=0.36$, $p=0.03$; $r=0.34$, $p=0.06$ controlling for pre-ART HIV RNA and CD4 count). Correlations were not observed between: i) CA-DNA and other HIV-specific T-cell responses ($p>0.1$), nor ii) between the slopes of decay of any HIV-specific T-cell responses and CA-RNA, plasma HIV-RNA, %$CD38^{+}HLA\text{-}DR^{+}$ T-cells, age, or PD-1 expression.

Conclusions: Overall, HIV-specific T-cell responses were stable, with long half-lives, and differed by sex. Females also had higher magnitudes of HIV-specific T-cell responses. This result may help explain previous findings that females have a lower residual viremia in this cohort. Higher CA-DNA at study entry correlated with slower rates of decay in Nef/Tat/Rev-specific T-cell responses on long-term ART, consistent with a level of ongoing recognition of infected cells.

PP 4.1

Baseline inducible HIV P24 is associated with viral control during interferon-α monotherapy with ART interruption

L.J. Montaner[1], L. Azzoni[1], E. Papasavvas[1], P. Tebas[2], K. Mounzer[3], B. Howell[4], D. Holder[4], N. Chomont[5], L. Kuri Cervantes[2], M. Betts[2]

[1] Wistar Institute, Philadelphia, USA, [2] University of Philadelphia, Philadelphia, USA, [3] Philadelphia FIGHT, Philadelphia, USA, [4] Merck, Inc., West Point, USA, [5] Université de Montréal, Montreal, Canada

Background: Pegylated (peg) IFNα monotherapy after ART interruption Results: in increased HIV control in association with NK cell activation. The relationships between inducible or other HIV proviral reservoir measurements with subsequent time to rebound during ART interruption and Peg IFN-a monotherapy are unknown.

Methods: 13 individuals randomized to arm 1 of NCT02227277 (HIV VL < 50 copies/ml on ART, CD4 count > 450/μl) receiving 1 μg/kg of peg-IFNα-2b (Pegintron, Merck) for 20 weeks, interrupting ART at week 4 and resuming it upon reaching VL > 50 copies/μl, or at week 20.

P24 SIMOA (iP24) was measured in CD4+ T cells cultured for 16-hour with medium or PMA/Ionomycin using single-molecule array (SIMOA). Intact, 5′ defective, 3′ defective and total proviral DNA were measured by Accelevir, Inc. on CD4+ T cells; Integrated HIV proviral DNA was assessed using Alu-gag RT-PCR on CD4+ T cells. Time to viremia: days to first VL > 50 copies/ml off ART.

HIV-specific responses: a) T cell - 6-hour cultures of PBMC with 15-mer gag peptides. B) NK ADCC: 4-hour co-cultures with anti-HIV sera with gp120-coated CEM NKres targets. Multicolor flow cytometry was used to assess HIV-specific degranulation cytokine production.

Associations were tested with Pearson or Spearman tests, and linear regression models.

Results: 12 participants became viremic during ART interruption; one remained suppressed and was imputed to week 20. ip24 was positively associated ($p<0.05$) with time to viremia (effect estimate 0.362; $p=0.029$; Adj $R2 = 0.305$), first detected VL (Fig 1), and Fc receptor-dependent expression of intracellular MIP1β in $CD56^{dim}$/$CD57^{neg}$ NK cells, but not with T-cell responses to Gag peptides. Proviral measures were correlated to each other, as expected, but not with time to viremia or first VL measured.

Conclusions: In vitro-inducible HIV p24, but not total proviral HIV DNA measures were associated with viral control during peg-IFNα-2b monotherapy. Unexpectedly, measures of latent reservoirs were not associated with time to viremia. The immune correlates measured are consistent with a contribution of NK cell ADCC and chemokine responses to viral control off ART.

PP 4.2

Enhancement of antiviral CD8 T-cell responses and complete remission of metastatic melanoma in an HIV-1 infected subject treated with pembrolizumab

O. Blanch-Lombarte[1], C. Gálvez[1], B. Revollo[2], E. Jiménez-Moyano[1], J.M. Llibre[2], J. Dalmau[1], D.E. Speiser[3], B. Clotet[2], J.G. Prado[1], J. Martinez-Picado[1]

[1] AIDS Research Institute, IrsiCaixa, Badalona, Spain, [2] Infectious Diseases Department, University Hospital 'Germans Trias i Pujol', Badalona, Spain, [3] Department of Oncology, University of Lausanne, Lausanne, Switzerland

Background: Pembrolizumab is an immune checkpoint inhibitor against programmed cell death protein-1 (PD-1) approved for therapy in metastatic melanoma. PD-1 expression is associated with a diminished functionality in HIV-1 specific-CD8+ T cells. It is thought that PD-1 blockade could contribute to reinvigorate antiviral immunity and reduce the HIV-1 reservoir.

Methods: Upon metastatic melanoma diagnosis, an HIV-1 infected individual on stable suppressive antiretroviral regimen was treated with pembrolizumab. A PET-CT was performed before and one-year after pembrolizumab initiation. We monitored changes in the immunophenotype and HIV-1 specific-CD8+ T-cell responses during 36 weeks of treatment. Furthermore, we assessed changes in the viral reservoir by total HIV-1 DNA, cell associated-HIV-1 RNA, and ultrasensitive plasma viral load.

Results: Complete metabolic response was achieved after pembrolizumab treatment of metastatic melanoma. Activated CD8+ T-cells expressing HLA-DR+/CD38+ transiently increased over the first 9 weeks of treatment. Concomitantly, there was an augmented response of HIV-1 specific-CD8+ T cells with TNFα production and poly-functionality, transitioning from TNFα to an IL-2 profile. Besides, a transient reduction of 24% and 32% in total HIV-1 DNA was observed at weeks 3 and 27, respectively, without changes in other markers of viral persistence.

Conclusions: These data demonstrate that pembrolizumab may enhance the HIV-1 specific-CD8+ T-cell response, marginally affecting the HIV-1 reservoir. A transient increase of CD8+ T-cell activation, TNFα production, and poly-functionality resulted from PD-1 blockade. However, the lack of sustained changes in the viral reservoir suggests that viral reactivation is needed concomitantly with HIV-1-specific immune enhancement.

Conflict of interest: O.B.L, C.G, E.J.M, J.G.P have received grants from MSD (Merck Sharp and Dohme España, S.A.), during the conduct of the study. B.C has received grants from ViiV and Gilead; grants and personal fees from MSD, outside the submitted work. J.M.P has received grants and personal fees from MSD, during the conduct of the study; grants and personal fees from ViiV Healthcare, Gilead Sciences, Janssen, and AbiVax, outside the submitted work. All remaining authors have declared no conflicts of interest.

PP 4.3

Characterising antibody responses in ART-treated individuals

A. Wilson[1], Y. Ren[2], E. Stevenson[2], R.B. Jones[2], R. Lynch[1]

[1] George Washington University, Washington, USA, [2] Weill Cornell School of Medicine, New York, USA

Background: Although suppression of HIV is now possible through antiretroviral therapy (ART), ART-treated individuals must maintain therapy to avoid rebound from a viral reservoir. Strategies to limit or clear this reservoir are urgently needed. Research has shown that individuals infected for longer prior to receiving ART harbor greater reservoir diversity, but may also have higher anti-HIV antibody titers. The roles that infection length and viral diversity play in the humoral response must be further studied to inform approaches to clearing infection. Here, we aim to clarify a role, if any, for autologous antibodies in these treatments by characterizing their function in individuals with different lengths of infection.

Methods: Plasma was collected from 8 HIV+ males on ART. Bulk IgG was isolated and normalized concentrations were tested for binding to gp41 and gp120 proteins. IgG was then tested for breath and potency of neutralization against a global HIV panel and autologous outgrowth viruses derived from each individual.

Results: Binding against gp41 was highly correlated with gp120, and binding titers were correlated with potency against the global panel. On average, participants exhibited low-potency neutralization of 8/12 global panel viruses. Interestingly we did not observe potent autologous neutralization of outgrowth virus, and in fact 2 of 8 people harbored completely resistant virus. 5 of the 8 individuals had a documented HIV-negative date, and therefore antibody functionality could be correlated to estimated length of infection before ART. We observe that length of infection is not correlated with autologous neutralization, but we do observe a trend toward more potent neutralization of the global panel by individuals with longer active infections.

Conclusions: Our findings agree with published studies of untreated individuals that length of infection is related to neutralization breadth. By contrast, we found that duration of ART treatment was not associated with differences in neutralization – either heterologous or autologous. Overall, these data suggest that the inducible reservoir is relatively resistant to autologous antibodies whether the individuals are ART-suppressed early or late after diagnosis.

PP 4.4

Th2 cytokines are associated with higher levels of intact proviruses on ART

J. Cyktor[1], H. Mar[2], G. Laird[3], R. Bosch[2], A. Martin[3], J. Eron[4], B. Macatangay[1], D. Mcmahon[1], R. Gandhi[5], J. Mellors[1]

[1] University of Pittsburgh, Pittsburgh, USA, [2] Harvard University, Boston, USA, [3] Accelevir Diagnostics, Baltimore, USA, [4] University of North Carolina, Chapel Hill, USA, [5] Massachusetts General Hospital, Boston, USA

Background: Th2 cytokines, such as interleukin (IL)-4 and IL-13, regulate humoral immunity, promote production of neutralizing antibodies, and can suppress Th1 and Th17 responses by upregulating repressors of interferon (IFN)-γ and IL-17 transcription. Th2 cytokines could affect the persistence of HIV-1 on ART, but their effects on the HIV-1 reservoir have not been defined.

Methods: Participants in AIDS Clinical Trials Group study A5321 who initiated ART during chronic HIV-1 infection with sustained virologic suppression had measurements of plasma HIV-1 RNA by

single copy assay (SCA), total HIV-1 DNA and cell-associated RNA (CA-RNA) from PBMC, intact proviral DNA (IPDA) from CD4+ T cells, and plasma levels of IL-1RA, IL-4, IL-10, IL-11, IL-13, CCL-22, and TGFβ. Exploratory cross-sectional analyses assessed the relationship between these cytokines and measures of HIV-1 persistence.

Results: 98 participants (21 females) were evaluated with a median (IQR) age of 46 years (37, 53) and 6.7 (4, 8) years on suppressive ART. Plasma levels of IL-4 were associated with the levels of intact proviral DNA (r=0.37, p=0.009), and the other main Th2 cytokine, IL-13, showed a trend towards a positive association with intact proviral DNA (r=0.26, p=0.07) (Table 1). There was also a trend towards an association of IL-4 levels with SCA HIV-1 RNA (r=0.2, p=0.06) but not total HIV-1 DNA (r=0.14, p=0.18) or CA-RNA (r=0.16, p=0.14). IL-1RA, IL-10, IL-11, IL-13, CCL-22, and TGFβ were not significantly associated with plasma SCA (N=95), total HIV-1 DNA (N=95), CA-RNA (N=90), or IPDA (N=48).

Conclusions: Th2 cytokines are associated with a higher frequency of intact proviral HIV-1 DNA but not total HIV-1 DNA, whereas cytokines including IL-10 were not associated with intact or total HIV-1 DNA. There was a weaker association of IL-4 with residual viremia, which likely arises from cells with intact proviruses. This demonstrates the value of measuring intact proviral HIV-1 DNA when evaluating the relationship between immune responses and the HIV-1 reservoir. While the mechanistic link between IL-4 and IL-13 levels and cells carrying intact proviruses is undefined, these findings suggest that the dampening effect of Th2 cytokines on Th1 and Th17 responses could promote persistence of the HIV-1 reservoir.

PP 4.5

Marker of gut damage REG3α and microbial translocation are associated with integrated HIV DNA in CD4 T cells during early HIV infection

S. Isnard[1], F.P. Dupuy[1], J. Lin[1], B. Fombuena[1], R. Ramendra[1], J. Ouyang[1], B. Lebouché[1], C. Costiniuk[1], C. Tremblay[2], J.P. Routy[1]

[1] McGill University, Montreal, Canada, [2] Université de Montréal, Montreal, Canada

Background: The impediment to an HIV cure lies in the early formation of HIV reservoirs, mostly consisting of CD4 T-cells carrying integrated HIV DNA in their genome. In addition to HIV itself, gut damage and microbial translocation fuels inflammation and HIV reservoir size that is maintained in people living with HIV (PLWH) receiving antiretroviral therapy (ART). Herein, we assessed the association of different gut damage and microbial translocation markers with integrated HIV DNA levels in early and chronically infected PLWH.

Methods: Blood from ART-naïve PLWH in early infection (less than 6 months after the estimated date of infection, ART-, n=33) or people in chronic infection ART-treated (ART+, n=29) was collected. Integrated HIV DNA was measured by nested qPCR in sorted CD4 T-cells. Gut damage markers regenerating islet-derived protein 3α (REG3α), intestinal fatty-acid binding protein (I-FABP), and soluble suppressor of tumorigenicity 2 (sST2), as well as the bacterial marker lipopolysaccharide (LPS) were measured in plasma by ELISA. The fungal translocation marker β-D-glucan (BDG) was measured in plasma with the Fungitell® assay.

Results: Participants of the ART- group were infected for a median of 97 days with a viral load (VL) of 4,12 $\log_{10}$ copies/mL. Participants in the ART+ group were infected for a median of 16 years, treated for 13 years, and had VL <50 copies/mL. In ART- PLWH, levels of REG3α, but not I-FABP nor sST2, correlated with VL (r=0.31, p=0.007) and integrated HIV DNA (r=0.42, p=0.02). None of these gut damage markers were associated with integrated HIV DNA in the ART+ group. In all study participants, REG3α correlated with LPS (r=0.24, p=0.004) and BDG (r=0.18, p=0.03). LPS and BDG were associated with integrated HIV DNA levels (r=0.35, p=0.04 and r=0.54, p=0.001 respectively) in ART- only but not in ART+.

Conclusions: The gut damage marker REG3α was associated with VL and integrated HIV DNA in ART-naïve but not ART+ PLWH, in association with microbial translocation of bacterial and fungal products. In addition to HIV itself, gut damage and microbial translocation fuel HIV reservoir size in early HIV infected individuals. These findings may help developing strategies to dampen reservoir formation in acute HIV infection.

PP 4.6

BCL-2 antagonism sensitises CTL-resistant HIV reservoirs to elimination *ex vivo*

R.B. Jones[1,3], Y. Ren[1], S.H. Huang[1], S. Patel[2], W.C. Alberto[1], D. Magat[1], D. Ahimovic[1], A.B. Macedo[3], A. Bosque[3], C.M. Bollard[2]

[1] Infectious Diseases Division, Weill Cornell Medicine, New York, Ny, USA, [2] Childrens National Medical Center, Washington, DC, USA, [3] Dept of Microbiology, Immunology, and Tropical Medicine, George Washington University, Washinton, DC, USA

Background: Curing HIV infection will require the elimination of a reservoir of infected $CD4^+$ T-cells that persists despite HIV-specific cytotoxic T-cell (CTL) responses. While viral latency is a critical factor in this persistence, recent evidence also suggests a role for intrinsic resistance of reservoir-harboring cells to CTL killing. This resistance may have contributed to negative outcomes of clinical trials, where pharmacologic latency reversal has thus far not resulted in reductions in HIV reservoirs.

Methods: RNA-seq was performed to identify genes that were differentially expressed in peptide-pulsed $CD4^+$ T-cells that survived co-culture with HIV-specific CTL clones. Protein-level expression of putative drivers of resistance was measured in HIV-Gag^+ cells reactivated from long-term ART-suppressed individuals by 'HIVFlow cytometry', which targets two epitopes of Gag. HIV eradication (HIVE) assays were used to test *ex vivo* elimination of reservoir-harboring cells. Briefly, HIV-specific T-cells were co-cultured with *ex vivo* $CD4^+$ T-cells, and changes in infected-cell frequencies were measured by droplet-digital-PCR - total HIV DNA, and QVOA - intact-inducible reservoir.

Results: We identified 1,061 genes that were differentially expressed between survivor cells (HIV peptide-pulsed) and bystander cells (non-pulsed) following co-culture with a peptide-specific CTL clone (Padj < 0.05) - implicating 14 significantly enriched pathways (Ingenuity Pathway Analysis). Amongst these, we prioritized the pro-survival factor BCL-2 - over-expressed in surviving cells - for further study. We confirmed by flow cytometry that BCL-2^{hi} cells preferentially survived peptide-pulsed CTL-killing assays using *ex vivo* $CD4^+$ T-cells. By 'HIVflow', we observed that the inducible HIV-reservoir in ARV-treated individuals was disproportionately present in BCL-2^{hi} $CD4^+$ T-cells, following *ex vivo* re-activation. In HIVE assays, treatment with the BCL-2 antagonist 'ABT-199' alone was not sufficient to drive reductions in *ex vivo* viral reservoirs, when tested either alone, or with the latency reversing agent (LRA). However, the triple combination of LRAs, HIV-specific T-cells, and ABT-199 uniquely enabled reductions in *ex vivo* reservoirs (p<0.01).

Conclusions: Our Results: provide rationale for targeting resistance to CTL as a novel component of therapeutic strategies for reducing HIV reservoirs. We highlight BCL-2 antagonism as one potential target for such approaches, and present additional differentially expressed genes and pathways associated with survival for further study.

PP 4.7

PD-1+ CD4 T cells are associated with HIV reservoir size and impaired function of T follicular helper cells in children and young adults on long-term viral control

S. Rinaldi[1], V. Dinh[1], S. Pallikkuth[1], L. De Armas[1], R. Pahwa[1], N. Cotugno[2], E. Nastouli[3], C. Foster[4], P. Palma[2], S. Pahwa[1]

[1] Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, USA, [2] Bambino Gesu Children's Hospital, Rome, Italiy, [3] UCL Great Ormond Street Institute of Child Health, London, UK, [4] Imperial College Healthcare NHS Trust, London, UK

Background: Curative strategies for HIV will need to eliminate the replication competent latent reservoir. CD4 T cells expressing Immune Checkpoint molecules (ICP) have been shown to preferentially harbor latent, replication-competent HIV. T follicular helper (Tfh) cell subset of CD4 T cells are critical for B cell differentiation. Here a cohort of HIV vertically infected children and young adults under durable viral control (PHIV) were investigated for CD4 ICP, immune activation (IA) markers and function in relation to HIV reservoir size.

Methods: 40 PHIV (4-19yrs age) who started ART <2 years of life and had undetectable viremia (<50 HIV copies/ml) for the past 5 years, were enrolled in 7 European research centers. HIV-DNA copies per million peripheral blood mononuclear cells (PBMC) were measured by real-time PCR. Flow cytometry was used to investigate CD4 T cells for 1) co-expression of PD1 with IA (ICOS, CD38, Ki67 and HLA-DR) or ICP (TIGIT, LAG3, TIM3 and CTLA4) and 2) intracellular cytokine production (IL2, IFNg, TNFα, IL21) after stimulation with ENV peptides. Pearson correlations and 2 group comparisons were performed using the Mann-Whitney T Test. P value<0.05 was considered significant.

Results: Total PD1+ CD4 T cells positively correlated with HIV-DNA (r=0.46) as did CD4 T cells co-expressing PD1 with other ICP or IA (table 1 below). We then divided our cohort based on HIV-DNA distribution into those with high (4th quartile) and low (1st quartile) HIV-DNA. We found that PD1+ CD4 T cells co-expressing IA or ICP were higher in participants with high HIV-DNA (table 1). PD1+ CD4 T cells also showed negative correlation with ENV antigen activated Tfh expressing CD40L (r=-0.41, p<0.05) with selective induction of IL2 (r=0.47, p<0.05), a cytokine that is inhibitory for Tfh.

Conclusions: This study suggests that PD-1 expression on CD4 T cells is associated with dysfunctional T:B cell interaction in response to HIV antigens, and supports the association of PD1 expression on CD4 T cells with size of viral reservoirs in vertically HIV infected children and young adults under long-term viral control.

PP 4.8

Restriction factor expression in HIV-1 vertically-infected children

D. Copertino Jr[1], M. Bortlik[2], B. Phillip[2], G. Beckerle[1], C. Ormsby[3], M. Rosenberg[4], R.A.S. Raposo[2], D. Nixon[1], M. De Mulder Rougvie[1]

[1] Weill Cornell Medicine, New York, USA, [2] Department of Microbiology, Immunology and Tropical Medicine, George Washington University, Washington, USA, [3] Centre for Research in Infectious Diseases, National Institute of Respiratory Diseases, Mexico City, Mexico, [4] Pediatric Infectious Diseases Department, Jacobi Medical Center, Bronx, USA

Background: Over three million children are estimated to live with HIV-1 worldwide and around 300,000 are newly infected every year. Most children acquire HIV-1 perinatally from their infected mothers. Restriction factors are cellular proteins that effectively inhibit HIV-1 replication. Little to no data has been reported on the expression of host restriction factors in children. Because of this gap in knowledge, we hypothesized that restriction factor expression might correlate with viral load, CD4 counts and T cell activation and could be a predictive variable of disease progression in children.

Methods: Thirty perinatally infected children were selected from the Jacobi Cohort and a total of 121 specimens, ranging from 2-26 years of infection, were analyzed. CD4+ T cells were enriched from total PBMCs and gene expression of APOBEC3A (A3A), A3C, A3G, A3H, SAMHD1, ISG15, CDKN1A, MX2, TRIM5α and SLFN11 were determined by quantitative real-time PCR. Cell surface expression of BST-2/tetherin and T cell activation of CD4+ T cells were analyzed by flow cytometry.

Results: After adjusting for gender and age, BST-2/tetherin expression on CD4+ T cells showed a significant positive correlation with viral load (P=0.0006) and CD4+ T cell activation (P<0.0001), and a negative correlation with CD4+ T cell counts (P=0.0008). In contrast to the expected association of restriction factor variables with markers of T cell activation, the expression of SAMHD1 was correlated negatively (P=0.046).

Conclusions: We conducted a longitudinal analysis of host restriction factor expression on perinatally infected HIV-1-positive children and found that BST-2/tetherin was strongly associated with disease progression. However, SAMHD1 deviates from the rest of the genes screened and is negatively associated with activation markers. Our Results: suggest an important role of these genes/proteins in the pathogenesis of HIV-1 in children, and should therefore be further explored. Understanding and harnessing the power of innate human antiviral responses bring us closer to finding a cure for HIV-1.

PP 4.9

HIV-infected macrophages evade NK cell-mediated killing while driving inflammation

K. Clayton[1], H. Stuart[1], G. Mylvaganam[1], A. Villasmil Ocando[1], M. Maus[2], B. Walker[1]

[1] Ragon Institute of MGH, MIT and Harvard, Cambridge, USA, [2] Massachusetts General Hospital, Cambridge, USA

Background: The primary targets for HIV infection are CD4+ T cells, however macrophages also become infected and persist despite antiretroviral therapy, suggesting evasion of immune responses. Our previous work shows that while HIV-infected macrophages are recognized by cytolytic CD8+ T lymphocytes (CTL), killing is inefficient due to resistance to CTL-derived granzymes. This poor killing delays CTL detachment from its target, causing hypersecretion of CTL-derived cytokines that propagate inflammation, emphasizing the need for rapid killing and release of effector-target contacts to limit inflammation. Thus, we hypothesized that cells with greater cytolytic potential compared to CTL, such as NK cells, would be able to rapidly kill HIV-infected macrophages while limiting excessive inflammation.

Methods: To test this hypothesis, innate interactions between ex vivo NK cells and autologous HIV-infected macrophages or CD4+ T cells were assessed via flow cytometry-based recognition and killing assays. To characterize the potential for antibody-dependent cellular cytotoxicity (ADCC), HIV envelope expression on macrophages was characterized by flow cytometry, imaging flow cytometry, and confocal microscopy using HIV-specific antibodies, and HIV-specific CAR T cells were used to confirm envelope accessibility on target cells. Finally, ADCC responses against infected CD4+ T cells and macrophages were assessed via flow cytometry.

Results: Despite similar levels of total recognition of HIV-infected CD4+ T cells and macrophages (degranulation and TNF-αproduction), NK responses to macrophages were significantly skewed towards non-cytolytic, cytokine production (p<0.0001), which was associated with poor elimination (p<0.0001). HIV antibody-based detection confirmed that envelope was transiently expressed on the macrophage cell surface, and recognition of infected macrophages by HIV-specific CAR T cells was comparable to that of CD4+ T cells, suggesting that HIV envelope is equally accessible on both cell types. ADCC enhanced NK cell responses to both cell types, however, total responses to macrophages were significantly lower compared to that of CD4+ T cells (p<0.001 for 3BNC117 and p<0.05 for PGT121).

Conclusions: Together, these data suggest HIV-infected macrophages employ a unique mechanism to evade cytolytic recognition by NK cells while preserving pro-inflammatory cytokine responses, emphasizing the need to develop alternative strategies to eliminate infected macrophages.

PP 4.10

A modified TZM-bl assay (MoCo-TZA) documents replication-competent HIV in circulating monocytes despite 2 years of antiretroviral therapy initiated early during acute infection

B. Mitchell[1], E. Laws[1], R. Paul[2], S. Vasan[3], E. Kroon[3], V. Valcour[4], S. Spudich[5], C. Shikuma[1], J. Ananworanich[3], L. Ndhlovu[1]

[1] University of Hawaii, Honolulu, USA, [2] Missouri Institute of Mental Health, St. Louis, USA, [3] South East Research Collaboration

in HIV, Bangkok, Thailand, [4]Memory and Aging Center, San Francisco, USA, [5]Yale University, New Haven, USA

Background: Determination of replication-competent HIV (rcHIV) in monocytes (MO) in the setting of ART-suppressed HIV, remains a topic of intense interest, particularly due to the continued presence of neurocognitive decline as well as in our pursuit for an HIV cure.

Methods: PBMCs from 8 acute HIV infection (AHI) adults were cell-sorted using flow-cytometry to obtain ultra-pure MO and CD4 T-cells confirmed by subsequent flow analyses. Using a novel modified TZM-bl co-culture assay (MoCo-TZA), frequencies of cells producing rcHIV (IUPM) were calculated. Total p24 associated with rcHIV produced by MO or CD4 T-cells was quantified using a relative light unit to rcHIV-associated p24 standard curve. The infectious potential (IP), was calculated as a ratio of total rcHIV-associated p24 and IUPM. Non-parametric tests assessed group comparisons and correlations.

Results: All AHI adults were males with a median age of 32 years. All initiated ART early near time of infection, were virally-suppressed, and had cognitive performance (NP) scores measured after 2 years of ART. IUPM in MO were detectable in all 8 donors (median IUPM=2.62) with a median IP of 0.44. IUPM in CD4 T-cells were detectable in 7 of 8 donors (median IUPM=2.26) with a median IP of 0.21. Unlike differences previously presented (IAS 2019) in a chronic HIV cohort (median IUPM: MO=1.32, CD4 T-cell=7.22; p=0.004), IUPM in AHI adults were similar between cell types (p=0.959). MO trended toward having higher IP as compared to CD4 T-cells in AHI (p=0.161). No relationships were seen between IUPM or IP and NP scores in both cell types.

Conclusions: Despite early ART in AHI, circulating MO retain and produce rcHIV and may contribute to long term neurocognitive decline or brain atrophy in subsequent years and pose a challenge for HIV remission efforts. Monitoring and targeting MO HIV persistence are warranted.

PP 4.11

B cells mediate R5-Tropic HIV infection of CCR5^{neg} naive CD4 T cells

A. Gerberick, N. Sluis-Cremer, P. Piazza, D. Delucia, C. Rinaldo, G. Rappocciolo

University of Pittsburgh, Pittsburgh, USA

Background: Naive CD4 T (T_N) cells are an important reservoir of latent, replication-competent HIV. T_N isolated from peripheral blood are resistant to direct infection with R5-tropic HIV in vitro due to negligible expression of CCR5. Paradoxically, R5-tropic virus has been isolated from T_N cells from HIV-infected individuals on antiretroviral therapy. We assessed B cell and dendritic cell (DC) mediated *trans* infection of R5-tropic HIV to T_N cells in the absence of global T cell activation.

Methods: Total CD4 T cells, CD4 T_N cells, B cells and monocytes were purified from PBMCs of 10 seronegative donors by magnetic microbead separation. B cells were activated by CD40L and IL4, and DCs were differentiated from monocytes by GM-CSF and IL4. B cells and DCs were pulsed with 10^{-3} moi R5-tropic HIV_{BaL} and cultured with T_N or total CD4+ T cells. We also directly exposed T_N or total CD4 T cells to 10^{-1} moi of HIV_{BaL} (i.e., *cis* infection). Viral replication was also assessed before and after stimulation of co-cultures with anti-CD3/CD28 Ab or PMA/PHA. We quantified total HIV DNA in the T_N and total CD4 T cell populations from 2 HIV nonprogressors (NPs).

Results: We detected p24 in the B cell-T_N co-cultures indicative of productive infection, but not in the DC-T_N co-cultures. In contrast, both B cells and DCs could efficiently HIV *trans* infect total CD4 T cells. As expected, T_N were refractory to *cis* infection with HIV_{BaL}. Phenotypic analysis of the T_N cells revealed that they maintained a CCR5^{neg} phenotype. B cell-T_N co-cultures exposed to anti-CD3/CD28 Ab or PHA/PMA resulted in high-levels of p24 production, whereas no virus expression was recovered from the DC-T_N co-cultures. Furthermore, we quantified the HIV DNA reservoir in T_N and total CD4 T cells isolated from 2 NPs, and detected HIV DNA in the total CD4 T cells but not in the T_N of both NPs.

Conclusions: B cells efficiently *trans* infect CCR5^{neg} T_N cells with R5-tropic HIV_{BaL}. No HIV DNA was detected ex vivo in CD4 T_N cells from NPs. B cell-mediated HIV *trans* infection of CD4 T_N cells could be a key mode to establish early HIV reservoir.

PP 4.12

Harnessing the specialised effector function of FcRγ$^-$ NK cells to control HIV-1 infection

R. Anderko, C. Rinaldo, R. Mailliard

University of Pittsburgh, Pittsburgh, USA

Background: In addition to causing natural killer (NK) cell dysfunction, chronic HIV-1 infection contributes to the expansion of a rare population of NK cells deficient in FcRγ (FcRγ$^-$). However, the implications of this phenomenon in HIV-1 infection remain to be elucidated. As NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) has been implicated in phenotypes of viral control, we assessed the ability of NK cells from participants with chronic HIV-1 infection to perform ADCC, with particular attention directed toward the FcRγ$^-$ population.

Methods: To evaluate ADCC activity, we used a flow cytometry-based cytotoxicity assay to quantify true lytic responses and NK cell activation through CD107a mobilization and intracellular IFNγ expression. Freshly isolated NK cells from randomly selected HIV-1 seropositive participants on suppressive antiretroviral therapy in the Multicenter AIDS Cohort Study (MACS) were exposed to opsonized and non-opsonized target cells. We calculated the specific elimination of antibody-coated targets based on the relative proportions of opsonized to non-opsonized target cells.

Results: NK cells from virally suppressed HIV-1$^+$ MACS participants effectively mediate the specific elimination of targets in an antibody-dependent manner, indicated by an NK cell dose-dependent decrease of opsonized target cells. A comparison of FcRγ$^+$ and FcRγ$^-$ NK cells reveals a functional bias of the FcRγ$^-$ subset toward antibody-dependent reactivity, with increased degranulation, as determined by cell surface CD107a, and IFNγ expression in the presence of antibody-coated target cells.

Conclusions: FcRγ$^-$ NK cells from virally suppressed HIV-1$^+$ individuals demonstrate superior ADCC activity. While the physiological relevance of this expanded population in HIV-1 infection is poorly understood, our data highlight the potential for harnessing the natural power of these specialized effector cells in the design of novel therapeutic strategies.

Sessions 5 and 7: Human studies and drug development I and II

OP 5.2

HIV post-treatment control despite plasma viral evolution and dual infection

J. Li[1], B. Etemad[1], G. Namazi[1], Y. Wen[2], N. Jilg[3], E. Esmaeilzadeh[1], X. Zhang[4], R. Sharaf[1], Z. Brumme[5], M. Kearney[6]

[1] Brigham and Women's Hospital, Harvard Medical School, Boston, USA, [2] China Medical University, Shenyang, China, [3] Massachusetts General Hospital, Harvard Medical School, Boston, USA, [4] Beijing Friendship Hospital, Beijing, China, [5] Simon Fraser University, Burnaby, Canada, [6] Frederick National Laboratory for Cancer Research, Frederick, USA

Background: HIV post-treatment controllers (PTCs) serve as models for sustained HIV remission. They frequently have early HIV rebound before viral control and subsequent periods of intermittent low-level viremia. Little is known about the viral composition during these periods of viremia.

Methods: We extracted plasma HIV RNA from PTCs and post-treatment non-controllers (NCs) from AIDS Clinical Trials Group (ACTG) analytic treatment interruption (ATI) trials. Single-genome sequences (SGSs) of HIV-1 pol were obtained at pre- and multiple post-ATI time points. Sequence analysis included viral genetic diversity by average pairwise distance (APD), root-to-tip distances, percent of HLA-escape mutations, and panmixia testing.

Results: Despite low plasma viremia, >1200 SGSs were obtained for 20 PTCs and 13 NCs. Early after ATI, chronic-treated NCs had the highest levels of plasma HIV diversity while viral diversity was limited for both early-treated PTCs and NCs. Over time, increasing viral diversity was detected in almost all PTCs, but rates of diversification were significantly slower in PTCs compared to NCs (median 0.05% vs 0.27% per year, p=0.007). PTCs maintained viral control despite evidence of viral evolution. This included increasing root-to-tip distances of HIV sequences by phylogenetic analysis over time for all PTCs, divergent population structures by the panmixia test in 73% of PTCs, and accumulation of HLA escape mutations in a subset. The proportion of HLA-escape mutations were common in HIV sequences from PTCs and not significantly different than NCs (47% vs 59%, p=0.16). Unexpectedly, the presence of dual HIV infections (populations of HIV variants with ≥5% sequence divergence) was detected in the plasma SGSs for 3 PTCs and for none of the NCs. In two participants, dual infection was detected at the early ATI time point with one variant becoming dominant over time. One individual was found to have an apparent superinfection with a late post-ATI viral rebound of a second HIV variant before subsequently regaining HIV control.

Conclusions: PTCs exhibit sustained HIV remission despite evidence of slow plasma viral diversification and evolution. The detection of dual HIV infection in a subset of PTCs suggests the presence of an antiviral response that can control a diverse viral population.

OP 5.3

Optimisation of Smac mimetics as HIV-1 latency reversing agents

L. Pache[1], P. Teriete[1], M.D. Marsden[2], A.M. Spivak[3], D. Heimann[1], A.J. Portillo[1], V. Planelles[3], J.A. Zack[2], N.D.P. Cosford[1], S.K. Chanda[1]

[1] Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA, [2] University of California, Los Angeles, Los Angeles, USA, [3] University of Utah School of Medicine, Salt Lake City, USA

Background: Current 'shock and kill' approaches seek to purge the latent viral reservoir by treating patients with therapeutics that activate latently infected cells and lead to their subsequent elimination. Due to adverse effects or a lack of efficacy of most latency reversing agents (LRA), at this time it is far from clear what type of treatments would make up an effective shock therapy. We have previously demonstrated the ability of molecules belonging to the class of Smac mimetics to reverse HIV latency through activation of the non-canonical NF-κB pathway. We now report the development of a novel Smac mimetic compound optimized for LRA activity.

Methods: An evaluation of a diverse set of Smac mimetic compounds revealed significant differences in their potency as LRA. Correlating the biochemical and pharmacological properties of these molecules with their activities has allowed us to develop novel compounds in this class that exhibit significantly greater potency and efficacy with regards to HIV latency reversal. We have profiled one of these compounds and assessed its *in vivo* activity in a BLT humanized mouse model of HIV latency.

Results: Our studies led to the development of SBI-0953294, a Smac mimetic compound with significantly improved LRA activity. The EC_{50} of SBI-0953294 was improved >100-fold compared to SBI-0637142 described in earlier studies. *In vivo* assessment of the compound in mice demonstrated superior bioavailability in the absence of observed toxicity including general T cell activation. Importantly, an evaluation of SBI-0953294 in a humanized mouse model of HIV infection demonstrated an *in vivo* proof of concept for HIV latency reversal by this compound.

Conclusions: The molecular class of Smac mimetics, and in particular SBI-0953294, shows promising *in vivo* bioavailability and efficacy, as well as an acceptable safety profile. Our Results: identify molecules belonging to this de-risked class of compounds as promising candidates for the development of shock-and-kill strategies to eliminate the latent HIV reservoir.

OP 5.4

HIV particles expressed in semen under INSTI-based suppressive therapy are largely myeloid cell-derived and exhibit widely diverse genotypes

J. Johnson[1], D. Anderson[2], J.F. Li[1], A. Santos Tino[3], J. Politch[2], J. Lipscomb[1], J. Defelice[4], M. Gelman[4], K. Mayer[4]

[1] CDC, Atlanta USA, [2] BU School of Medicine, Boston, USA, [3] DESA Group, Atlanta, USA, [4] Fenway Institute, Boston, USA

Background: As use of integrase strand transfer inhibitors (INSTI) in first-line HIV therapies increases and formulations are being developed for PrEP/PEP, assessing virus dynamics in semen after initiating INSTI-based regimens is timely. Additionally, evaluating the genetic variance of HIV in semen relative to peripheral blood can inform interpretation of transmission events.

Methods: Men living with HIV on INSTI-based regimens for >6 months (n=15, group A) or ≤6 months (n=5, group B) provided semen, blood and rectal swabs for virologic examination. Treatment history, viral load (VL), and duration of infection were obtained for each participant. The day following specimen collection, HIV particles were isolated from blood and seminal plasmas by immunocaptures that targeted cell surface proteins embedded in virus envelopes indicative of cell source. HIV captures with amplifiable RNA were sequenced in pol/RT to assess genetic diversity both within and between semen and blood, and in integrase and 3'PPT to examine for INSTI resistance mutations.

Results: Five of 15 men in group A had blood plasma virus at <40 copies/mL, the remaining had undetectable VL. All five men in group B had undetectable peripheral blood VL. Durations of infection were >3-28 years. HIV captures yielded amplifiable RNA from the semen of 7/15 men in group A and 3/5 men in group B. Three participants each from groups A and B had captured blood virus that could be sequenced. Two participants in each group had seminal virus with RT M184V, K103N, Y181C or G190A mutations not found in blood virus. No INSTI resistance was identified. The immunocaptures identified seminal virus to be primarily CD3- and CD14+, CD16+, CD11c+, Iba1+ or CD45RA+, indicative of diverse macrophage and resident dendritic cell sources. These seminal viruses had genetic distances of up to 9% (29 of 318 nucleotides differed) between compartments over the RT region analyzed (Figure).

Conclusion: Suppressive therapy significantly prevents HIV sexual transmission (Undetectable=Untransmittable). Under therapy, however, immunocaptured seminal variants had evidence of large genetic distances (>2%) relative to blood viruses. This has implications for assessing genotypic linkage if insufficient suppression leads to their transmission. Detectable seminal virus was not associated with INSTI resistance.

Conflicts of interest: None

OP 5.5

Impact of anti-PD-1 and anti-CTLA-4 on the HIV reservoir *in vivo*: AMC-095 Study

Sharon R. Lewin[1,8], Thomas A. Rasmussen[1], Laskhmi Rajdev[2], Ajantha Rhodes[1], Ashanti Dantanarayana[1], Surekha Tennakoon[1], Socheata Chea[1], Danielle Rigau[3], Shelly Lensing[4], Rachel Rutishauser[5], Sonia Bakkour[6], Michael Busch[6], Dirk P. Dittmer[7], Steven Deeks[5], Christine Durand[3]

[1] Peter Doherty Institute for Infection and Immunity, University of Melbourne and Royal Melbourne Hospital, Melbourne, Australia, [2] Department of Medicine, Albert Einstein College of Medicine, New York, USA, [3] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, [4] Department of Biostatistics, University of Arkansas for Medical Biosciences, Arkansas, USA, [5] Department of Medicine, University of California San Francisco, San Francisco, California, USA, [6] Vitalant Research Institute, San Francisco, California, USA, [7] Department of Microbiology and Immunology, University of North Carolina, USA, [8] Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Australia

Background: Antibodies to PD-1 and CTLA-4 may perturb HIV persistence during antiretroviral therapy (ART) by reversing HIV-latency and/or boosting HIV-specific immunity. We tested this hypothesis in a prospective multi-center clinical trial of individuals on ART who had cancer and received singleimmune checkpoint blockade (ICB) therapy with nivolumab (anti-PD-1) or combination therapy with nivolumab and ipilimumab (anti-CTLA-4).

Methods: This is a substudy of the AIDS Malignancy Consortium-095 Study. ART-suppressed HIV-infected participants with advanced malignancies were assigned to nivolumab (anti-PD-1) 3 mg/kg or 240 mg every two weeks with or without ipilimumab 1 mg/kg (anti-CTLA-4) every 6 weeks. In samples obtained at baseline, within 24 hours and 7 days after the first and fourth dose of ICB and again after multiple cycles, we quantified cell-associated unspliced (CA US) HIV-RNA and CA HIV-DNA. Plasma HIV-RNA was quantified during the first cycle of ICB using replicate testing on the Aptima HIV-1 Quant assay. Quantitative viral outgrowth to estimate the frequency of replication-competent HIV was done at baseline and during ICB for a subset of participants. Changes from baseline, including the difference between those on single compared to dual ICB, were tested using non-parametric and parametric statistics (as appropriate) and repeated-measures analysis of variance.

Results: Forty participants were included, 36 males and 4 females. Of those, 33 received anti-PD-1 alone and 7 received anti-PD-1 plus anti-CTLA-4. At baseline, median age was 53.0 (IQR 47.0-58.5) and CD4 count was 315 (IQR 227-465). Whereas CA US HIV-RNA did not change from baseline in those receiving anti-PD-1 alone, we detected a median 1.44 fold-increase (IQR 1.16–1.89) within 24 hours of the first dose in participants on combination ICB (P=0.031). This increase was also significantly higher compared to the corresponding change from baseline in those on anti-PD-1 alone (P=0.025). We detected no changes in the level of HIV DNA or the frequency of cells containing replication-competent HIV, but in two of two individuals on combination ICB with samples available for QVOA, the frequency of replication-competent HIV decreased.

Conclusions: Dual ICB with anti-PD-1 and anti-CTLA-4 induced a larger increase in CA-US HIV RNA than anti-PD-1 alone and may potentially impact the frequency of cells containing replication-competent HIV.

OP 5.6

Intact proviral DNA levels decline in people with HIV on antiretroviral therapy

J. Cyktor[1], R. Gandhi[2], R. Bosch[3], H. Mar[3], G. Laird[4], B. Macatangay[1], J. Eron[5], R. Siliciano[6], D. Mcmahon[1], J. Mellors[1]

[1] University of Pittsburgh, Pittsburgh, USA, [2] Massachusetts General Hospital, Boston, USA, [3] Harvard T.H. Chan School of Public Health, Boston, USA, [4] Accelevir Diagnostics, Baltimore, USA, [5] University of North Carolina, Chapel Hill, USA, [6] Johns Hopkins School of Medicine, Baltimore, USA

Background: The intact proviral DNA assay (IPDA) is a new, more-specific ddPCR-based measure of the replication-competent HIV reservoir. Little is known about whether intact proviral DNA levels decline over time on ART and whether the levels correlate with other measures of HIV persistence or with immune activation.

Methods: Participants in ACTG A5321 with chronic HIV and virologic suppression on ART had the following measurements performed on blood samples: intact proviral DNA (IPD), total proviral DNA (sum of defective, hypermutated, intact proviruses), total HIV DNA by qPCR, cell-associated HIV RNA (CA-RNA), plasma HIV RNA single copy assay (SCA), T cell activation, and inflammation (including IL-6, IP-10, sCD14, sCD163). Testing was performed at median of 7.1 yr after ART initiation (time-point 1) and again a median of 3.7 yr later (time-point 2).

Results: Fifty participants (26% female) were evaluated. Intact proviral DNA levels declined significantly between time point 1 (n=50) and time point 2 (n=48): median 57 and 41 copies/million CD4-cells, respectively; p<0.001 (Figure). By contrast, total proviral DNA was stable: median 551 and 580 copies/million CD4-cells, respectively. Estimated half-life of decline for intact proviral DNA (n=44 participants) was 6.5 yrs (95% CI 4.5, 11.2), whereas half-life for total proviral DNA was 22.9 yrs (95% CI, 11.1 to 60.9). Six participants had decline in IPD to undetectable levels. Higher on-ART IPD levels correlated with higher on-ART total HIV DNA (r=0.48), higher CA-RNA (r=0.46), higher SCA (r=0.39) (time-point 1; all p-values ≤0.005). No associations were seen between on-ART IPD levels and on-ART T cell activation or inflammation.

Conclusions: In people on long-term ART, intact proviral DNA levels decline significantly (half-life 6.5 yr), whereas total proviral DNA remains stable (half-life 22.9 yr). A subset of individuals had a decline in intact proviral DNA to undetectable levels. The overall decline in intact proviruses implies that cells containing replication-competent proviruses are being lost. Defining the mechanisms involved should inform strategies to accelerate HIV reservoir depletion. The more dynamic nature of the intact proviral landscape, compared with total proviral HIV DNA, supports the use of the IPDA to assess the impact of interventions targeting the HIV reservoir.

Conflict of interest: Dr. Greg Laird is cofounder and an employee of Accelevir Diagnostics, a company which makes the intact proviral DNA assay used in this study.

OP 7.2

Plasma and antibody glycomic biomarkers of time to HIV rebound and viral setpoint

M. Abdel-Mohsen[1], L. Giron[1], E. Papasavvas[1], L. Azzoni[1], K. Mounzer[2], J. Kostman[2], I. Sanne[3], C. Firnhaber[4], Q. Liu[1], L. Montaner[1]

[1] Wistar Institute, Philadelphia, USA, [2] Philadelphia FIGHT, Philadelphia, USA, [3] University of the Witwatersrand, Johannesburg, South Africa, [4] University of Colorado School of Medicine, Aurora, USA

Background: HIV cure research urgently needs to identify pre-Analytic Treatment Interruption (ATI) biomarkers of viral rebound and viral setpoint to mitigate the risk of ATI and accelerate the development of a cure. We previously reported that galactosylated, bulk IgG glycans negatively correlate with cell-associated HIV DNA and RNA during antiretroviral therapy (ART). We hypothesized that this and other plasma glycomic traits can predict time to viral-rebound and viral setpoint upon ART-cessation.

Methods: Using capillary electrophoresis and a lectin microarray, we profiled the circulating glycomic signatures (plasma and bulk IgG) of two geographically-distinct cohorts: (1) Philadelphia Cohort – 24 HIV-infected, ART-suppressed individuals who had participated in an open-ended ATI study without concurrent immunomodulatory agents. This cohort had a wide distribution of viral-rebound times (14 to 119 days; median=28) and viral-setpoints (median=13,675 copies/ml). (2) Johannesburg Cohort, serving as a validation cohort of 23 HIV-infected, ART-suppressed individuals who had participated in a two-week ATI. Cox proportional-hazards model and log-rank test were used for statistical analyses.

Results: Higher pre-ATI levels of the IgG glycan, G2, were significantly associated with a longer time-to-viral-rebound (hazard ratio (HR)=0.12, P=0.05) in the Philadelphia cohort. G2 glycan levels were significantly lower at viral-rebound (P=0.02) and viral set-point (P=0.009) compared to their pre-ATI levels. In addition to G2, we identified several predictive glycomic traits in plasma, e.g., levels of FA2BG1, a non-sialylated, core-fucosylated glycomic trait, strongly associated with a longer time-to-viral-rebound (HR=0.023, P=0.05), whereas FA2G2S1, a sialylated glycomic trait, strongly associated with a shorter time-to-viral-rebound (HR=24.1, P=0.028). Additionally, pre-ATI plasma glycomic signatures associated with lower viral setpoint, e.g., T-antigen (Galβ1-3GalNAc) (r=0.75, P=0.0007), or higher viral setpoint, e.g., polylactosamine (r=-0.58, P=0.01). These Results: were validated in the Johannesburg validation cohort.

Conclusions: We describe first-in-class, non-invasive, plasma and bulk IgG glycomic biomarkers that strongly inform time-to-viral rebound and viral setpoint upon ART-cessation, in two geographically-distinct cohorts. Our data warrant further validation in larger cohorts and examination of potential functional significance. These pre-ATI biomarkers may enhance the conduct of ATI and accelerate the path to develop effective HIV curative strategies.

OP 7.3

Single cell RNA-seq identifies host genes that correlate with HIV-1 reservoir size

R. Thomas[1], A. Waickman[2], P. Ehrenberg[1], A. Geretz[1], M. Eller[1], S. Tovanabutra[1], J. Ananworanich[1], N. Chomont[3], J. Currier[2], N. Michael[1]

[1] US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, USA, [2] Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, USA, [3] Université de Montréal, Faculty of Medicine, Montreal, Canada

Background: Variations in genotype and expression of host genes are well established to impact HIV-1 susceptibility and disease progression in antiretroviral therapy (ART)-naive individuals. Host variation that influences viral reservoir size or reactivation during ART has not been studied using genome-wide methods and has the potential to significantly advance HIV cure research. We hypothesized that host cellular factors impact the size of latent viral reservoirs, independent of differences in viral load in acutely-treated people living with HIV.

Methods: We used next-generation sequencing technologies to investigate cell-specific, transcriptome-wide host gene expression variation influencing the size of viral reservoirs. Peripheral blood mononuclear cells were available from 14 virally suppressed individuals with variable levels of HIV viral DNA following 48 weeks of therapy initiated during Fiebig III acute infection. We performed single cell RNA sequencing (scRNA-Seq) analysis using the 10x Genomics Chromium Single Cell Immune Profiling platform to investigate transcriptional and lymphocyte clonal variation at single-cell resolution. Sequence analysis was performed using the 10x Genomics Cell Ranger pipelines and the R package Seurat. In parallel, multiparameter flow cytometry was performed to identify immune cell populations.

Results: High quality gene expression and paired immune receptor sequence data was captured from approximately 63,000 cells across 14 donors. We detected 22 major cell populations within this dataset, including all major T cells, NK cells, monocytes, naïve/memory B cells, plasmablasts, DCs and megakaryocytes. The abundance of populations as quantified by scRNA-Seq correlated directly with their frequency as defined by concurrently performed flow cytometric analysis. The monocyte and CD8+ effector/memory T cell populations had the highest number of significantly differentially expressed genes between the high and low reservoir groups.

Conclusions: Despite a lack of any apparent active viral replication or ongoing adaptive immune response to infection at the time of analysis, significant differences in the gene expression profile of multiple cell subsets were noted between donors with variable reservoir size following 48 weeks of ART and are currently being validated. Host cellular proteins that impact HIV-1 reservoir size have potential therapeutic implications, including the design of new strategies for improving clinical treatment, vaccine development and intervention.

OP 7.4

Sex differences in the latent reservoir of virally suppressed HIV-1 infected individuals living in Rakai, Uganda

T.C. Quinn[1], J. Prodger[2], A.M. Capoferri[3], K. Yu[2], S.J. Reynolds[1], J. Kasule[4], D. Serwadda[4], E. Scully[3], K.J. Kwon[3], A.D. Redd[1]

[1] National Institutes of Health, Baltimore, USA, [2] Western University, London, Canada, [3] Johns Hopkins School of Medicine, Baltimore, USA, [4] Rakai Health Sciences Program, Kalisizo, Uganda

Background: Understanding the establishment and maintenance of the latent viral reservoir (LVR) is critical to developing an HIV cure. Despite evidence of sex differences in HIV pathogenesis, the impact of biological sex on the LVR is not clear.

Methods: Blood was collected from HIV-positive, ART-suppressed (<40 copies/ml for >1 year) adults in Rakai, Uganda (n=57 females, n=33 males). LVR size was estimated in resting CD4 T cells (rCD4) using (1) quantitative viral outgrowth assay (QVOA, measuring inducible replication competent provirus) and (2) qPCR for a 103bp region of *gag* that is universal across major HIV-1 subtypes (total HIV DNA). Regression analyses were used to compare LVR size between sexes and identify clinical correlates of LVR size.

Results: Females and males were similar in age, subtype, nadir CD4, pre-ART viral load, and duration of infection and suppression; however, females had higher CD4 counts and CD4/CD8 ratios at the time of LVR quantification (p<0.01). Compared to males, females had a lower frequency of viral outgrowth (0.4 vs 1.1 IUPM, p<0.01; Figure A), despite having a trend towards an increased frequency of rCD4 containing HIV DNA (3.38 vs 3.08 HIV *gag*+ $\log_{10}$copies/10^6 rCD4, p=0.1; Figure B). Among females, age and duration of suppression correlated negatively with LVR size, while nadir CD4 correlated positively. In contrast, in males, LVR size correlated negatively with CD4/CD8 ratio (all p<0.05).

Conclusions: Females had a reduced frequency of viral outgrowth, despite having a trend towards higher levels of HIV DNA. Reduced viral outgrowth among females could be due to an increased proportion of defective provirus or reduced reactivation of intact provirus. As over half of individuals living with HIV are female, further studies investigating sex-based differences in LVR size, composition, and reactivation are warranted.

OP 7.5

Clones of HIV-infected cells are widely distributed in T cell subsets in blood and in anatomic tissues

F. Maldarelli[1], M. Gozoulis[1], X. Wu[2], L. Perez[3], R. Gorelick[4], C. Lange[1], S. Hill[1], J. Virga[1], T. Uldrick[5], R. Yarchoan[6], S. Hughes[1]

[1] CCR/NCI, Frederick, USA, [2] Leidos Inc., Peoria, USA, [3] VRC/NIAID, Bethesda, USA, [4] Leidos, INC, Peoria, USA, [5] CCR/NCI, U Washington, USA, [6] CCR/NCI, Bethesda, USA

Background: HIV persistence during combination antiretroviral therapy (cART) prevents viral eradication. HIV infected cells undergo

clonal expansion during cART; clones arise in infected T cells, but their distribution in subsets and tissues remains unclear. We investigated the distribution of clonally expanded cells in T cell subsets in peripheral blood lymphocytes (PBL) and in tissues obtained at autopsy.

Methods: We studied 5 participants undergoing prolonged cART (HIV RNA < 50 c/ml). In three otherwise healthy participants, PBL samples were sorted into naïve, central-transitional memory (CTM), and effector memory (EM) subsets. In two additional individuals with cancers [primary effusion lymphoma (PEL), adenocarcinoma], we obtained samples at autopsy. HIV integration sites were determined for cell subsets and for the individual with PEL, while HIV pro-pol single genome sequences (SGS) were obtained for the individual with adenocarcinoma.

Results: Clones of infected cells were widely distributed in the PBL subsets and anatomic locations. In the T cell subsets, 1205 integration sites were recovered; 8-33% were in clones that were in CTM and EM. In the individual with PEL, 391 integration sites were obtained from lymph node, lung, spleen, and testes; 72 of the integration sites (18.4%) were in clones. Clones were detected within individual tissues, but were also present across tissues, in both local (lung and draining lymph node), and distant tissues (lung, spleen, testes) sites; infected cells were also present in the effusion. HIV was integrated in many host genes, including BACH2 and STAT5B, genes in which proviruses are associated with clonal expansion and persistence. In the individual with adenocarcinoma, 183 SGS were recovered. HIV sequences were genetically highly diverse, but identical sequences, indicating possible cell clones, were present within and across tissues. Individual metastases all contained HIV infected cells.

Conclusions: Clones of HIV infected cells were widely, but not uniformly, distributed. Tumors contained infected cells. Analyzing neoplasms contributes to our understanding of their role in the immune response during cART.

OP 7.6

Tissue-specific differences in the mechanisms that govern HIV latency in blood, liver, gut and genital tract in ART-suppressed women

S. Moron-Lopez[1], G. Xie[2], P. Kim[3], J. Wong[1], J. Price[4], N. Elnachef[4], R. Greenblatt[4], P. Tien[1], N. Roan[2], S. Yukl[1]

[1] University of California San Francisco, SFVAMC, San Francisco, USA, [2] University of California San Francisco, Gladstone Institutes, San Francisco, USA, [3] San Francisco VA Medical Center, San Francisco, USA, [4] University of California San Francisco, San Francisco, USA

Background: Sex-specific differences affect various aspects of HIV infection. An HIV functional cure will likely require a major reduction of infected cells from most tissues. However, few studies have quantified HIV in tissues from women. Here, we measured the HIV transcription profile and HIV reservoir size in blood, liver, gut, and genital tissues from ART-suppressed women.

Methods: PBMC, liver, gut (ileum, colon, rectosigmoid), genital tract biopsies (cervix, endometrium), and endocervical curettage (ECC) samples were collected from 5 women with plasma HIV RNA<200 copies/ml (median 10.4 years). Total and intact (IPDA) HIV DNA and levels of read-through, initiated, 5'elongated, polyadenylated, and multiply-spliced HIV transcripts were measured by ddPCR. Phenotyping of immune cells was conducted by CyTOF.

Results: HIV DNA was detected in all tissues, with levels being comparable between the gut, liver and genital tract. Intact proviruses were detected in PBMC, ileum, colon and cervix. HIV transcriptional initiation (HIV RNA/provirus) tended to be higher in PBMC and endometrium than in ileum, colon, rectosigmoid, cervix, and ECC (all p=0.06), and higher in rectum than either ileum or colon (p=0.06). Likewise, levels of elongated HIV RNA per provirus were comparable in PBMC and endometrium, but higher than the gut and cervical samples (p=0.06). Polyadenylated HIV RNA were detected in PBMC from all 5 individuals but were rarely detected in the tissues. Multiply-spliced HIV RNA were detected in PBMC from 2 of 5 individuals, but not detected in any tissue. The phenotypes of CD4+ T cells were distinct between PBMC, genital tract, and gut.

Conclusions: The gut, liver, and genital tract are all sites of HIV persistence in women. The female genital tract contains a large pool of HIV-infected cells like the gut. HIV-infected cells in the blood and endometrium showed higher levels of HIV transcription per provirus, while much lower levels were observed in the gut, cervix and liver. These Results: suggest tissue-specific differences in the mechanisms that govern HIV latency, with greater suppression of HIV transcription in most tissues than blood. Therapies aimed at disrupting latency, such as latency-reversing or -silencing, will be required to penetrate multiple tissues and affect different blocks to HIV transcription.

PP 5.7.1

From reservoirs to the real world: a framework for integrating behavioural and social sciences research into biomedical HIV cure-related research

K. Dube[1], J.D. Auerbach[2], M.J. Stirratt[3], P. Gaist[4]

[1] UNC Gillings School of Global Public Health, Chapel Hil, USA, [2] School of Medicine, University of California San Francisco, San Francisco, USA, [3] Division of AIDS Research, National Institute of Mental Health, National Institutes of Health, Bethesda, USA, [4] Office of AIDS Research, Division of Program Coordination, Planning, and Strategic Initiatives, Office of the Director, NIH, Bethesda, USA

Background: Tremendous progress has been made in pre-clinical and clinical research toward an HIV cure. As cure regimens progress through the translational research pathway, increasing attention should be paid to their socio-behavioural components, as their effectiveness will be determined by whether and how people with HIV take them up. The importance of integrating behavioural and social sciences research (BSSR) into the biomedical cure research agenda has been well-articulated, but a specific framework for identifying priorities within that agenda has not.

Methods: We applied to HIV cure research Gaist and Stirratt's functional framework, which describes four key domains of HIV BSSR: 1) basic BSSR (understanding basic behavioural and social factors), 2) elemental BSSR (advancing behavioural and social interventions), 3) supportive BSSR (strengthening biomedically-focused clinical trials), and 4) integrative BSSR (building multi-disciplinary approaches for real-world implementation).

Results: We identified emerging topics from the application of the framework to HIV cure-related research and research priorities under each domain. Basic BSSR priorities include research on the optimal language to define HIV cure research, understanding community perceptions and knowledge, framing of expectations, construction and management of HIV-related identifies, and social meanings of curing HIV. Elemental BSSR priorities include counseling and support interventions related to analytical treatment interruptions, behavioural risk-reduction strategies, and HIV stigma reduction interventions. Supportive BSSR priorities include assessing the acceptability of HIV cure research approaches, capturing participant reports and experiences, ensuring adequate informed consent, and strategies to mitigate social impacts during trial participation. Integrative BSSR priorities include addressing features of real-world implementation of HIV cure research strategies, such as decision tools, equitable access, and cost-effectiveness.

Conclusions: BSSR can contribute an improved understanding of the social dimensions of HIV cure research and its eventual application. The functional BSSR framework provides a way to identify advances, gaps and opportunities in this arena, including how best to craft an integrated, multi-disciplinary approach at all stages of research to ensure the real-world applicability of any strategy that shows promise.

PP 5.7.2

Permanent control of HIV-1 pathogenesis in exceptional elite controllers: a model of spontaneous cure

C. Gálvez[1], C. Casado[2], M. Pernas[2], L. Tarancón-Diez[3], C. Rodríguez[4], R. Lorenzo-Redondo[5], E. Ruiz-Mateos[3], M. Salgado[1], C. Lopez-Galindez[2], J. Martinez-Picado[1]

[1]AIDS Research Institute IrsiCaixa, Badalona, Spain, [2]Centro Nacional de Microbiología. Instituto de Salud Carlos III, Madrid, Spain, [3]Institute of Biomedicine of Seville, Seville, Spain, [4]Centro Sanitario Sandoval, Madrid, Spain, [5]Northwestern University Feinberg School of Medicine, Chicago, USA

Background: Elite controllers (EC) represent a small subset of HIV-1-infected people able to spontaneously control viral replication. However, natural virological suppression and absence of immune dysfunction are not always long-term sustained. Exceptional EC (EEC) are HIV-1 subjects who maintain the EC characteristics without disease progression for more than 10 years.

Methods: We analyzed three EEC from the Sandoval Health Center in Madrid, diagnosed between 1988 and 1992, who without antiretroviral treatment have never shown signs of clinical progression. A comprehensive clinical, virological, and immunological study has been performed.

Results: The three EEC studied, diagnosed for more than 25 years, simultaneously exhibited previously described EC characteristics as ≥3 host protective alleles, low levels of total HIV-1 DNA (<20 copies/10^6 $CD4^+$ T-cells), absence of viral transcription, without evidence of replication-competent viruses (<0.025 Infectious Units Per Million). This was consistent with high levels of defective genomes, and strong cellular HIV-1-specific immune response with a high poly-functionality index (>0.50). Inflammation levels of EEC (measured as plasma levels of hsPCR, β2-microglobulin, D-Dimer, IL-6 and sCD163) were similar to HIV-1 negative donors. Remarkably, they showed 8-fold lower genetic diversity (<0.01 s/n) in env gene than transient EC, and an exceptional lack of viral evolution.

Conclusions: We postulate that these EEC should be considered unique cases of spontaneous functional HIV-1 cure. Low genetic diversity and lack of viral evolution distinguish these individuals from other EC. The combined non-functional HIV-1 reservoir, extremely low viral diversity and an HIV-1-specific immune response seems to be key to mimic these cases of spontaneous functional cure in future eradication strategies.

PP 5.7.3

Awareness of HIV cure-directed research among HIV clinic patients in Philadelphia

W. Freshwater[1], L.J. Montaner[2], B. Peterson[2], C. White[1], M. Lefae[3], C. Roebuck[4], N. Jones[5], D. Robinson[1], S. Johnson[6], E. Burton[6]

[1]BEAT-HIV CAB, Philadelphia, USA, [2]Wistar Institute, Philadelphia, USA, [3]AIDS Law Project, Philadelphia, USA, [4]Cornell University, Ithaca, USA, [5]Temple University, Philadelphia, USA, [6]Philadelphia FIGHT, Philadelphia, USA

Background: Understanding the awareness, perceptions, misperceptions and interest in HIV cure research is necessary to design meaningful content and effective delivery of educational messages targeting the community of potential participants.

Methods: The Delany BEAT-HIV Community Advisory Board conducted an anonymous survey of patients receiving HIV care from two clinics in the University of Pennsylvania Heath System during August, 2019. Patients were invited to complete the brief survey as they attended routine clinic visits. Responses were collected via a self-administered, web-based data capture tool. Survey questions were designed to assess awareness, general attitudes towards participation in HIV cure research, beliefs about a cure for HIV, and best ways to deliver information about cure research.

Results: A total of 102 patients completed the survey. Of these, 43% were between 31 to 50 years old and 48% were over 50; 72% were African American/black; and 76% were male. Overall, 77% had heard of cure research and 49% knew that cure research was being conducted in Philadelphia. While 49% reported being very willing to participate, 15% were not at all willing. Over one third (35%) believed that a cure for HIV already existed and 6% stated that they believed a cure for HIV would never be found. Overall, 87% reported that they wanted to receive more information about cure research. Most (75%) indicated that this information would be best communicated by their doctor while 14% suggested the use of web-based educational materials, and 4% endorsed community meetings.

Conclusions: This CAB initiated, clinic-based survey collected information on awareness, attitudes, and interest in research toward a cure for HIV. Willingness to participate in future HIV cure research was high and respondents expressed great interest in receiving more information about cure research. The most preferred method for information dissemination was through their doctor. Beliefs about an existing cure for HIV suggest the need for clear information regarding the current status of HIV cure research. These findings offer guidance for the development of community level educational activities regarding cure-directed research.

PP 5.7.4

Collaboration in community education: BEAT-HIV Community Engagement Group (CEG) and the HIV cure research education video series

L.J. Montaner[1], B. Peterson[1], W. Freshwater[2], C. White[2], M. Lefae[3], C. Roebuck[4], N. Jones[5], J. Shull[6], D.S. Metzger[7], J.L. Riley[7]

[1]Wistar Institute, Philadelphia, USA, [2]BEAT-HIV CAB, Philadelphia, USA, [3]AIDS Law Project, Philadelphia, USA, [4]Cornell University, Ithaca, USA, [5]Temple University, Philadelphia, USA, [6]Philadelphia FIGHT, Philadelphia, USA, [7]University of Pennsylvania, Philadelphia, USA

Background: This video project was created using the CEG model, a unique partnership among the BEAT-HIV CAB, Philadelphia FIGHT, and Wistar/Penn. Key goals of the CEG include advancing community engagement in HIV cure research and developing meaningful and relevant community education tools. Our video project aimed to demystify cure research and to initiate dialogue about perceptions and misperceptions of cure research. Awareness and interest in the HIV cure research agenda is growing, and the video project tells this story from the perspectives of PLWHIV, researchers/providers, and key stakeholders involved in advancing the search for a cure.

Methods: The CEG identified topics important to PLWHIV interested in cure trials and developed an RFP sent to video services. N=4 proposals were received and 3 invited to present to the CEG and Wistar/FIGHT Communications. The CEG selected the production team via deliberation. Two community meetings held at FIGHT introduced the project, engaged participants in the process, and solicited feedback about the series. A diverse group of CAB members, advocates, clinicians/investigators, and social workers were recruited to be interviewed (n=34), including 6 study participants (3 of whom were cure trial volunteers). The CEG reviewed draft videos through 3 rounds of edits to ensure inclusivity in the representation in terms of age, gender, race and ethnicity and balance across community and professional perspectives. Final CEG approval was achieved before release.

Results: 65 hours of video were recorded and edited to create four videos for release. The videos (3-8 min/ea., total 30 minutes) have been viewed 2000x since June 11, 2019. They include: 1) GAME CHANGERS (who/what is behind HIV cure research); 2) THE TOP TEN (items to consider (and ask about) when joining a study); 3) THE ART OF A.R.T. (analytical treatment interruption); 4) TIME. COMMITMENT. (researchers and study participants discuss what it takes to finish a trial).

Conclusions: The series provides a model of partnership between a traditional CAB, a community-based organization, and academia. The success of this project – process, development, and outcome – demonstrates the critical and creative impact of the CEG model of engagement to advance education about HIV cure research.

PP 5.7.5

Behind the scenes: clinical and rapid autopsy staff members' experiences and perceptions of HIV reservoir research at the end of life

K. Perry[1], J. Taylor[2], S. Concha-Garcia[3], S. Javadi[4], H. Patel[1], A. Kaytes[3], S. Little[3], D. Smith[3], S. Gianella[3], K. Dubé[1]

[1] University of North Carolina, Chapel Hill, USA, [2] HIV + Aging Research Project–Palm Springs, Palm Springs, USA, [3] University of California San Diego, La Jolla, USA, [4] University of California San Diego, Chapel Hill, USA

Background: Guidelines on HIV reservoir research at the end of life are emerging; yet little is known about the effects of such research on staff members involved. We assessed staff members' experiences, perceptions, ethical considerations, resilience, and coping mechanisms related to their involvement in the Last Gift study involving people with HIV at the end of life at the University of California San Diego.

Methods: We conducted focus groups with Last Gift clinical and rapid autopsy staff members to discuss issues involving ethics and resilience, among others. The sample consisted of 15 clinical and autopsy team members (66.7% females; 33.3% males) and was ethnically diverse (66.7% Caucasian/White, 6.7% African-American/Black, 20% Asian descent, 6.7% Hispanic and American Indian), with a range of experience in HIV research from 1 year to 30 years (mean: 7 years). Data analysis of focus groups is ongoing.

Results: Emerging themes included self-actualization, learning as an iterative process in clinical and rapid autopsy work, agency (control and choice regarding death and the dying process), interdependence between staff members themselves and staff members and study participants, research as an ennobling endeavor, ethics, vulnerability and resilience, as well as emotional compartmentalization and coping. In the Last Gift study, social science outcomes (e.g. relationship between clinical staff and participants/next-of-kin) and biomedical science outcomes (e.g. rapid autopsy and HIV reservoirs) were viewed as equally important, with meaningful community engagement being the sine qua non of the study. Constant communication and transparency are key to 'bridging' social sciences and biomedical outcomes and adhering to ethical standards. Ensuring that staff members are supported with healthy coping mechanisms, resiliency-building exercises, and at-work and at-home care networks allows for study sustainability and staff member fulfillment.

Conclusions: Understanding the complex operational and emotional mechanisms involved in conducting HIV reservoir research at the end of life is crucial to the study's success and sustainability. Scaling up the Last Gift study or reproducing it in another context will require an established research infrastructure with supported clinical and autopsy teams and trusted community buy-in channels for ethical engagement with people living with HIV towards the end of life.

PP 5.7.6

Virological impact of HCV elimination with DAAs in the HIV reservoir in HIV/HCV patients

M. Coiras, P. Martínez-Román, C. Crespo-Bermejo, A. Fernández-Rodríguez, V. Briz

Institute of Health Carlos III, Majadahonda, Spain

Background: Previously, our group observed an increase in HIV reservoir size in resting CD4 T cells (rCD4, CD25-CD69-HLA-DR-) from patients exposed to HCV (HIV+/HCV+ coinfected and HIV+/HCV– spontaneous clearers), compared to HIV+ monoinfected subjects. However, the effect of HCV elimination with direct-acting antivirals (DAAs) on HIV reservoir is unknown. Besides, information related to HIV reservoir in non-rCD4 in these patients remains uncertain. Objective: To assess the impact on HIV reservoir size of HCV elimination, through either spontaneous clearance or DAAs, in rCD4 and non-rCD4 T cells.

Methods: Longitudinal study (52 weeks follow-up) with 50 patients: 19 HIV+/HCV+, 16 HIV+/HCV– and 15 HIV+ subjects, all of them aviremic. Proviral DNA integrated was quantified in both rCD4 and non-rCD4 T cells with Alu-LTR qPCR. Paired samples were analysed using a generalized mixed linear model.

Results: A decrease in HIV proviral DNA was observed in rCD4 cells at the end of follow-up only in HCV exposed patients: HIV+/HCV– (Baseline: 1354.8 vs Final: 871.1 HIV DNA copies/10e6 cells; p=0.019); HIV+/HCV+ (693.8 vs 551.8; p=0.057); HIV+ (574.1 vs 291.9; p=0.142) (Fig. A). A significant increase in HIV reservoir was observed in non-rCD4 T cells from HIV+ patients (208.6 vs 831.4; p=0.036) (Fig. B). HCV-exposed patients also showed a tendency to an increase in HIV reservoir: HIV+/HCV– (591.0 vs 893.1; p=0.093) and HIV+/HCV+ (89.0 vs 827.7; p=0.113). Moreover, when comparing HIV reservoir size in rCD4 cells, HCV-exposed patients continue to show an increased size with respect to monoinfected patients (p=0.020) (Fig. C).

Conclusions: 1) At the end of the follow-up, rCD4 cells from HCV-exposed patients showed a decrease in the proviral DNA. However, HIV reservoir remained higher than HIV+ monoinfected group. 2) HIV reservoir in non-rCD4 cells leaned to an increase. 3) The larger size of HIV reservoir in HCV-exposed patients, even after eliminating HCV by DAAs, could be an obstacle to control and eliminate HIV infection in these patients

PP 5.7.7

HIV particles expressed in semen under INSTI-based suppressive therapy are largely myeloid cell-derived and exhibit widely diverse genotypes

J. Johnson[1], D. Anderson[2], J.F. Li[1], A. Santos Tino[3], J. Politch[2], J. Lipscomb[1], J. Defelice[4], M. Gelman[4], K. Mayer[4]

[1] CDC, Atlanta, USA, [2] BU School of Medicine, Boston, USA, [3] DESA Group, Atlanta, USA, [4] The Fenway Institute, Boston, USA

Background: As use of integrase strand transfer inhibitors (INSTI) in first-line HIV therapies increases and formulations are being developed for PrEP/PEP, assessing virus dynamics in semen after initiating INSTI-based regimens is timely. Additionally, evaluating the genetic variance of HIV in semen relative to peripheral blood can inform interpretation of transmission events.

Methods: Men living with HIV on INSTI-based regimens for >6 months (n=15, group A) or ≤6 months (n=5, group B) provided semen, blood and rectal swabs for virologic examination. Treatment history, viral load (VL), and duration of infection were obtained for each participant. The day following specimen collection, HIV particles were isolated from blood and seminal plasmas by immunocaptures that targeted cell surface proteins embedded in virus envelopes indicative of cell source. HIV captures with amplifiable RNA were sequenced in pol/RT to assess genetic diversity both within and between semen and blood, and in integrase and 3'PPT to examine for INSTI resistance mutations.

Results: Five of 15 men in group A had blood plasma virus at <40 copies/mL, the remaining had undetectable VL. All five men in group B had undetectable peripheral blood VL. Durations of infection were >3-28 years. HIV captures yielded amplifiable RNA from the semen of 7/15 men in group A and 3/5 men in group B. Three participants each from groups A and B had captured blood virus that could be sequenced. Two participants in each group had seminal virus with RT M184V, K103N, Y181C or G190A mutations not found in blood virus. No INSTI resistance was identified. The immunocaptures identified seminal virus to be primarily CD3- and CD14+, CD16+, CD11c+, Iba1+ or CD45RA+, indicative of diverse macrophage and resident dendritic cell sources. These seminal viruses had genetic distances of

up to 9% (29 of 318 nucleotides differed) between compartments over the RT region analyzed (Figure).

Conclusions: Suppressive therapy significantly prevents HIV sexual transmission (Undetectable= Untransmittable). Under therapy, however, immunocaptured seminal variants had evidence of large genetic distances (>2%) relative to blood viruses. This has implications for assessing genotypic linkage if insufficient suppression leads to their transmission. Detectable seminal virus was not associated with INSTI resistance.

PP 5.7.8

Rapid ART in blood donors with acute and recent HIV clade C infection in South Africa

E. Murphy[1], K. Van Den Berg[2], M. Vermeulen[2], S. Bakkour[3], M. Busch[3]

[1] UCSF and Vitalant Research Institute, San Francisco, USA, [2] South African National Blood Service, Johannesburg, South Africa, [3] Vitalant Research Institute, San Francisco, USA

Background: Blood donations in South Africa are tested in parallel for HIV antibody (Ab) and RNA using individual-donation nucleic acid testing (ID-NAT), allowing annual detection of ~60 Acute (RNA+/Ab–) and >400 Recent (RNA+/Ab+/LAg recent) HIV infections. We hypothesized that initiation of antiretroviral therapy (ART) in earlier Fiebig stages would correlate with smaller HIV reservoir size.

Methods: A prospective cohort study enrolled Acute and Recent HIV clade C infected blood donors from 2015-2017. HIV Ab (Abbott Prism), RNA (Grifols ID-NAT) and recency of infection < 195 days (Sedia limiting-antigen avidity (LAg)) were measured on samples taken at index donation and enrolment. Donors were referred rapidly for ART with RAL/TDF/FTC X 6 months followed by EFV/TDF/FTC. We measured plasma RNA using the Aptima HIV-1 Quant Assay (Hologic) with 5 replicates, cell-associated (CA) HIV RNA by qRT-PCR and total DNA by real-time nested PCR. After median treatment duration of 20 months, we compared HIV reservoir size between treatment initiated in Fiebig I-III vs. IV-VI using repeated measures analysis adjusting for baseline values.

Results: Cohort enrolment/ART initiation occurred at medians of 13/15 days after index donation. Longitudinal HIV reservoir data were available for 18 Fiebig I-III and 42 Fiebig IV-VI subjects. Median plasma RNA was 5.4 $\log_{10}$ copies/mL at enrolment, declined to 0.23 $\log_{10}$ copies/mL, did not differ by Fiebig stage (p=0.56) but was 0.31 $\log_{10}$ lower in females (p=0.02). Median CA RNA was 3.7 $\log_{10}$ copies/10^6 PBMC at enrolment, falling to 2.2 $\log_{10}$ copies/10^6 PBMC, and was 0.64 $\log_{10}$ higher in Fiebig IV-VI than Fiebig I-III treated-subjects (p=0.002). Median CA total DNA was 1.8 $\log_{10}$ copies/10^6 PBMC at enrolment, falling to 0.85 $\log_{10}$ copies/10^6 PBMC with no difference by Fiebig stage (p=0.95).

Conclusions: Among clade C HIV-infected donors initiated on ART within 195 days of infection, we observed a small impact of Fiebig I-III treatment on long-term reservoir as measured by lower CA RNA expression, and lower post-ART plasma HIV-1 (single copy assay) in women vs. men. This study demonstrated that a partnership between a national blood service and a treatment NGO can establish early treatment cohorts for subsequent HIV Cure research initiatives.

PP 5.7.9

HIV-1 in the latent reservoir is largely sensitive to circulating T cells

J. Warren, S. Zhou, Y. Xu, M. Moeser, J. Kuruc, C. Gay, D. Margolis, N. Archin, R. Swanstrom, N. Goonetilleke

University of North Carolina at Chapel Hill, Chapel Hill, USA

Background: In HIV infected individuals durably suppressed with ART (HIV+ART+), pre-existing variants in the HIV reservoir may limit CD8 T cell recognition and clearance of infected cells. While virus escape mutants have been reported in the HIV reservoir, the extent of escape in the latent reservoir is unclear.

Methods: HIV T cell responses were comprehensively mapped across the Clade B HIV proteome by ex-vivo IFN-g ELISpot in 25 ART-suppressed participants (range 1.6-30.09 years on ART, average=7.2). The quantitative viral outgrowth assay (QVOA) which provides a minimum estimate (infectious units per million, IUPM) of replication competent HIV was performed in resting CD4 T cells. p24 antigen positive wells in the QVOA were sequenced in 23/25 participants; in 7 participants p24 positive wells from serial QVOA were sequenced. A median of 25 outgrowth viruses (OGV) was obtained from each participant. All variant peptides corresponding to reactive T cell epitopes in OGVs were synthesized and tested for their impact on T cell response.

Results: HIV-specific T cell breadth ranged from 1-19 epitopes and magnitude ranged from 156-2855 SFU/M PBMCs across the cohort. Targeting of HIV-1 proteins was consistent with previous studies of untreated infection. HIV escape in this cohort was defined as a > 50% decrease in the average magnitude of the HIV-specific T cell response. We observed a cohort-level T cell escape frequency in the HIV reservoir of 32% (49/151 epitopes) and an average within-participant escape frequency of 34%. HIV-1 escape was most commonly observed in Pol, Env and Nef proteins, and in regions of higher entropy. Overall, these Results: were very consistent with previous studies of both the frequencies and patterns of HIV-1 escape in plasma viral RNA in untreated infection.

Conclusions: These data show that the majority (68%) of latent HIV viruses do not carry CD8 T cell escape mutants, suggesting that circulating T cells very likely afford some contribution in control of virus rebound. The implication of these Results: is that HIV-1 cure strategies to augment T cell immunity in HIV+ART+ can include designs that target existing, particularly low entropy T cell epitopes.

PP 5.7.10

Analytical treatment interruption and rearrangement of HIV-1 drug resistance mutations in peripheral reservoir

E. Bruzzesi[1], R. Scutari[2], M.C. Bellocchi[2], V. Spagnuolo[3], L. Galli[4], L. Carioti[2], M.M. Santoro[2], C. Alteri[2], F. Ceccherini-Silberstein[2], A. Castagna[1]

[1] Università Vita-Salute San Raffaele, Milan, Italy, [2] Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italiy, [3] Università Vita-Salute San Raffaele, Milano, Italy, [4] Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy

Background: The aim of the study was to evaluate the impact of short analytical treatment interruption (ATI) on archived major resistance mutations (MRMs).

Methods: 7 chronically HIV-1 infected patients (pts) with undetectable viremia (HIV-RNA<50cps/ml for ≥10 years) enrolled in the ATI study APACHE were tested for total HIV-DNA by ddPCR and pol sequences by Illumina MiSeq before ATI (T1), at viral rebound (VR), during ATI (T2) and at achievement of undetectable HIV-RNA after ART resumption (T3). These data were also collected in 7 ART-treated pts with HIV-RNA<50 cps/ml for ≥1year, enrolled in the MODAT study, at 3 similar time-points.

Intra-patient prevalence (IPP) of MRMs (Stanford 2018) and of APOBEC3G-related mutations were assessed at each time-point. Changes in MRM prevalence within and between APACHE and MODAT pts were analysed by Wilcoxon signed-rank and Mann-Whitney tests, respectively.

Results: APACHE pts experienced VR after ATI at a median time of 4 weeks and, after ART resumption, achieved HIV-RNA<50 cps/ml in 24 weeks. Median HIV-DNA cps/106CD4+T was 982 at T1, 1892 at T2, 992 at T3, with no significant changes overtime, despite a temporary expansion of peripheral HIV reservoir during ATI (P=0.368).

No difference was found in HIV-DNA (P=0.620 and P=0.530) at T1 and T3 between APACHE and MODAT patients.

At T1, 5/7 APACHE and 3/7 MODAT carried MRMs with a median IPP of 38.1 and 99.0, respectively.

In APACHE pts, MRMs persisted during ATI in 2/7 (both with IPP >99.0%) and in 4/7 (median IPP=53.1) at plasma level and in PBMCs, respectively. Post-ATI, HIV-DNA MRMs were found in 3/7 pts (with IPP of 1.4%, 99.6%, 99.8%). Comparing pre- and post-ATI HIV-DNA sequences, MRMs with a T1IPP <80% significantly decreased from pre- to post-ATI (IPP: 7.2 vs. 0; P=0.01), which occurred mainly in APOBEC-3G related MRMs. In MODAT pts, MRM prevalence did not change duringfollow-up, including MRMs with a T1IPP <80% (P=0.180).

Conclusions: This proof of concept study confirms that ATI does not affect the value of post-ATI peripheral HIV-DNA, but suggests that ATI may be associated with rearrangement of peripheral archived MRMs and, in some cases, with their complete reversal.

PP 5.7.11

Persistent HIV reservoir suppression by (-)-hopeaphenol, a plant-derived stilbenoid

I. Tietjen[1,2], Z. Haq[2], M. Naidu[2], J. Rivera-Ortiz[1], Y. Cai[1], K. Beattie[3], T. Rali[4], Z. Brumme[2], L. Montaner[1], R. Davis[3]

[1] Wistar Institute, Philadelphia, USA, [2] Simon Fraser University, Burnaby, Canada, [3] Griffith University, Brisbane, Australia, [4] University of Papua New Guinea, Port Moresby, Papua New Guinea

Background: While ART durably suppresses HIV replication, virus persists within cellular reservoirs. One experimental approach toward inactivating HIV reservoirs ('Block-and-Lock') involves reinforcing long-term and durable proviral latency, even in the presence of subsequent activation and/or latency-reversing stimuli. Here we identify and characterize (-)-hopeaphenol, a resveratrol tetramer, as a candidate Block-and-Lock agent.

Methods: 527 pure compounds from Compounds Australia were screened for ability to inhibit PMA-induced latency reversal in J-Lat cells containing an HIV-GFP provirus. Hopeaphenol (> 99% purity), the most active hit, was further assessed for antiviral activities in both CEM-GXR cells, which contain an LTR-driven GFP reporter, and PBMC. Ability of hopeaphenol to suppress latency reversal (inclusive of periods after wash-out) was assessed in J-Lat cells. Mechanisms of action were investigated via in vitro reporter constructs and enzyme inhibition.

Results: Hopeaphenol inhibited HIV replication with dose-dependence in infected CEM-GXR cells and PBMC (EC50s = 3.3 and 1.6 μM, respectively) without concomitant cytotoxicity. It also inhibited latency reversal induced by PMA, panobinostat, or TNFα in J-Lat cells (EC50s = 0.1 – 1 μM). Hopeaphenol blocked both NF-κB-dependent reporter expression and CDK9 enzymatic activity (respective IC50s = 9.4 and 0.2 μM). Following 24 h pre-treatment with hopeaphenol and 24 h compound wash-out and resting, J-Lat cells remained refractory to latency-reversal induced by PMA, panobinostat, or TNFα (EC50s = 2.1 – 3.5 μM). Finally, cells pre-treated with 10 μM hopeaphenol remained completely refractory to latency-reversal for up to 3 days post-washout.

Conclusions: We identify (-)-hopeaphenol as a potent HIV inhibitor with potential 'Block-and-Lock' activity that may support long-term, ART-free HIV remission strategies.

PP 5.7.12

Phorbol esters isolated from *Croton megalobotrys* reverse HIV latency *ex vivo*

I. Tietjen[1,2], K. Richard[2], D. Williams[3], J. Rivera-Ortiz[1], Y. Cai[1], A. Pagliuzza[4], N. Chomont[4], R. Andersen[3], L. Montaner[1], K. Andrae-Marobela[5]

[1] Wistar Institute, Philadelphia, USA, [2] Simon Fraser University, Burnaby, Canada, [3] University of British Columbia, Vancouver, Canada, [4] Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, Canada, [5] University of Botswana, Gaborone, Botswana

Background: Discovery of latency reversal agents (LRA) from natural products that are already administered to humans creates an opportunity to develop therapies with higher likelihoods of efficacy, safety, and successful clinical development. We previously described the traditional use of *Croton megalobotrys* bark ('Mukungulu') in Northern Botswana and showed that 'Mukungulu' crude extracts inhibit HIV-1 replication and promote latency reversal in vitro (PMIDs: 27350006, 28970153). Here we investigate whether 'Mukungulu' extracts and isolated pure compounds can target HIV replication and induce latency reversal in primary cells.

Methods: 'Mukungulu' extract was fractionated and iteratively tested for latency reversal in J-Lat cells containing an HIV-GFP provirus. 'Mukungulu' and isolated pure compounds were assessed for antiviral activity and cytotoxicity using in vitro-infected PBMC. Effects of extract and compounds on HIV latency reversal, as determined by the Tat/rev-Induced Limiting Dilution Assay (TILDA), in addition to cytotoxicity, T cell activation (CD69 expression), and CD4 downregulation, were measured in CD4+ T cells isolated from HIV-infected, ART-suppressed individuals. Reproducibility was assessed by repeat experiments using samples from multiple donors.

Results: 'Mukungulu' extract inhibited viral replication in PBMCs (EC50 = 2.3 μg/mL) with no concomitant cytotoxicity. Fractionation efforts led to isolation of 5 phorbol ester-class compounds (Namushen 1 – 5). Two purified Namushens at 0.03 μg/mL each reversed HIV latency in CD4+ T cells from two HIV-infected donors at 11.1 – 51.9% of the activity observed by 0.1 μg/mL PMA plus 1 μg/mL ionomycin (P+I). In contrast to P+I, 0.03 μg/mL of each Namushen did not affect cell viability (Average 88.6% viability vs. 77.2% for P+I, relative to unstimulated cells). However, cell activation and CD4 downregulation by 0.03 μg/mL of each Namushen resembled those of cells treated with P+I (CD69: Average 5.8-fold increased expression by Namushens vs. 5.5-fold by P+I; CD4: average 62.4% downregulation by Namushens vs. 60.1% by P+I; both relative to unstimulated cells).

Conclusions: We identify novel pure phorbol ester-class compounds, isolated from an African medicinal plant, with capacity to promote HIV latency reversal ex vivo. This in turn informs ongoing latency-reversal or 'shock-and-kill'-based therapeutic strategies in humans.

PP 5.7.13

Targeting HIV-1-driven aberrant transcription and proliferation

Y-C. Ho[1], Y.H. Yeh[1], K. Jenike[2]

[1] Yale University, New Haven, USA, [2] Johns Hopkins University, Baltimore, USA

Background: Despite effective antiretroviral therapy, HIV-1 persists in the latent reservoir as a major barrier to cure. More than 50% of the latent reservoir are maintained by clonal expansion. While HIV-1-infected cells undergo clonal expansion through antigen-driven proliferation and homeostatic proliferation, targeting these physiologic $CD4^+$ T cell homeostasis mechanisms will likely disrupt adaptive immunity. We propose to develop therapeutic strategies targeting HIV-1 integration site-related proliferation through preferential inhibition of HIV-1 transcription.

Methods: First, using a dual-reporter cell line system, we identified 4 FDA-approved drugs which can suppress HIV-1-GFP reporter expression without suppressing host gene expression. Second, we examined the effect of these HIV-1 suppressing agents in different cell line models (by measuring HIV-1-GFP reporter expression using flow cytometry) and in $CD4^+$ T cells from HIV-1-infected individuals (using cell-associated HIV-1 RNA qRT-PCR to quantify spliced and unspliced HIV-1 RNA). Third, to examine the effect of HIV-1-suppressing agents on HIV-1-driven aberrant transcription,

we examined the host gene RNA landscape at the integration site. Fourth, to examine which cellular pathways mediates HIV-1 suppression, we performed transcriptome analysis and differential gene expression analysis in HIV-1-GFP reporter cell line models and in $CD4^+$ T cells from HIV-1-infected individuals. Fifth, to examine the effect of HIV-1 suppressing agents on transcriptional landscape, we examined the intron retention landscape using IRFinder. Finally, to examine the impact of HIV-1-suppressing agents on the proliferation of HIV-1-infected cells, we examined the effect of HIV-1-suppressing agents on clonal expansion dynamics of HIV-1-infected $CD4^+$ T cells by measuring the frequency of cells harboring inducible HIV-1 upon ex vivo CD3/CD28-induced proliferation with and without HIV-1 suppressing agents.

Results: We identify a JAK1 inhibitor, filgotinib, which suppresses HIV-1 RNA splicing, HIV-1-driven aberrant host gene transcription through modulation of RNA splicing machinery. We found that filgotinib suppresses the clonal expansion of HIV-1-infected cells upon CD3/CD28-induced proliferation.

Conclusions: We identified the mechanism of filgotinib-induced suppression of HIV-1-induced aberrant host gene transcription. We found that filgotinib suppresses proliferation of HIV-1-infected cells ex vivo. HIV-1 suppressing agents serve as a new class of therapeutic agents which disrupt clonal expansion of HIV-1-infected cells.

PP 5.7.14

A mechanistic modelling platform for HIV cure drug development

Y. Cao, D. Rosenbloom, M. Ahamadi, S. Bae, R. Vargo

Merck & Co., Inc., Kenilworth, New Jersey, USA

Background: It is an open question whether proposed therapeutic strategies – such as reactivation of latent provirus ('flush') or immunotherapies – are capable of achieving functional cure of HIV. Mechanistic mathematical models that capture both within-host viral dynamics and immunologic control of HIV infection can integrate clinical data from trials of different therapeutic strategies, assess therapeutic response, and generate hypotheses in support of HIV cure drug development.

Methods: We built the Latent Viral Dynamics Modeling (LVDM) platform, based on recently developed mathematical models. Using Monolix, the LVDM was fitted to a large viral load (VL) dataset from clinical studies that together cover both long-term viral suppression during ART and analytical treatment interruption (ATI) (N = 896 participants in all). From estimated parameters, we sampled a virtual population (N = 2000) to run clinical trial simulations (CTS) of potential curative interventions. Predicted outcomes were summarized as the duration of viral suppression off therapy after ATI ('VSOT time').

Results: Despite the high nonlinearity of the LVDM, five parameters along with their inter-individual variabilities were estimated to capture individual-level viral dynamics during both ART and ATI phases (see Figure). CTS based on estimated parameters illustrated that the LVDM can reliably recapitulate and predict viral load responses during all phases of HIV infection. While VSOT time increases with duration of long-term ART, this effect reaches a plateau after a few years. A 'flush' mechanism added to ART, however, significantly increases VSOT time in simulations, and this effect does not reach a plateau even after 10 years of continuous treatment. Furthermore, it was observed that VSOT time varies widely across individuals regardless of the therapy tested.

Conclusions: Simulations quantify the potential role of long-term ART and 'flush' mechanisms in HIV cure. The LVDM platform can incorporate viral load measurements from heterogeneous datasets in order to fit population- and individual-level parameters. This platform will be continuously developed by incorporating new mechanisms in order to identify new targets and generate hypotheses about combination therapies, enabling design of HIV cure trials through CTS.

Conflict of interest: Authors are employed by Merck and work in commercial development of HIV therapies.

PP 5.7.15

In vitro demonstration of a potential role for STING agonist in HIV cure

A. Koblansky[1,2], S. Raines[2], J. Schawalder[1], D. Irlbeck[1], C. Galardi[1], J. Brehm[1], J. Ramanjulu[3], D. Margolis[2], H. Madsen[1]

[1] ViiV Healthcare, Chapel Hill, USA, [2] UNC HIV Cure Center, Chapel Hill, USA, [3] GlaxoSmithKline, Upper Providence, USA

Background: Latently infected cells represent the major obstacle for HIV cure. We envision that curing HIV will require a combination of mechanisms that are capable of reversing latency and clearing HIV infected cells. Among these interventions, modulation of innate immune responses that are typically involved in recognition of viruses could provide a new powerful tool. By targeting pattern recognition receptors (PRRs) we hope to induce a spectrum of immune responses that include activation of NF-κB to reverse HIV latency and induction of Type I Interferon via IRF3 to boost antiviral immunity. Among PRRs, the STimulator of Interferon Gene pathway 'STING' is a key immune sensor of DNA viruses and self-DNA that induces numerous genes that suppress pathogen replication and facilitate adaptive immunity. GSK has developed a novel series of potent and selective small molecule STING agonists (STINGa) that have been shown to activate both NF-κB and IRF3. We demonstrate in vitro that STING activation disrupts HIV latency and enhances HIV specific T cell response.

Methods: We stimulated human PBMCs, HIV-infected and healthy donors with the STINGa to demonstrate activation of specific signaling pathways important for latency reversal and CD8+ T cell activity.

Results: STINGa induced activation of both NF-κB and TBK1 signaling pathways in human PBMC. STINGa lead to the production of the antiviral Type I Interferon by phosphorylating TBK1. Stimulation with the STINGa resulted in a faster kinetic when compared to the STING endogenous ligand, cGAMP. In addition, STINGa stimulation lead to an enhancement HIV-specific CD8+ T cell activation and proliferation in a dose dependent manner. STINGa induced HIV latency reversal in HIV-infected donor CD4+ T cells.

Conclusions: This ex vivo study provides evidence that STINGa may offer a novel mechanism to both induce HIV latency reversal and enhance virus-specific immune responses.

PP 5.7.16

Enhancing antiretroviral drug penetration into lymph nodes through intramuscular and subcutaneous routes of administration in BALB/c mice

S. Dyavar

UNMC, Nebraska, USA

Background: Antiretroviral drugs (ARVs) are currently administered orally to HIV-infected persons to manage disease pathogenesis. HIV lymphoid tissue (LT) persistence, even during long-term suppressive antiretroviral therapy, has been associated with suboptimal concentrations of ARVs in LN. Alternative delivery methods to improve ARV pharmacokinetics (PK) in LN are under investigation. The comparative PK of ARVs in LN when administered through intramuscular (IM) and subcutaneous(SC)routes are unknown.

Methods: We determined the LN PK of rilpivirine (RPV), efavirenz (EFV), elvitegravir (EVG), cobicistat (COBI), tenofovir disoproxil fumarate (TDF), maraviroc (MVC) and tenofovir alafenamide (TAF), following IM, SC and oral routes of administration in mice (n=5). ARVs were administered as EFV/TAF (30/5 mg/kg), EVG/COBI (12/12 mg/kg) and MVC/TDF/RPV (60/60/5 mg/kg) combinations to determine LN PK. LN tissue was collected 4 hours after the 3rd daily dose and tissue concentrations were determined by LC-MS/MS.

Results: ARV LN concentrations were increased when administered through IM and SC routes versus oral. ARV LN concentrations were, IM, SC and oral, respectively: RPV, 3059 ± 706 vs 2522 ± 846 vs 1676 ± 531 ng/g; EVG, 3953 ± 1616 vs 3974 ± 1036 vs 726.5 ± 423; COBI, 1186 ± 799 vs 1806 ± 970 vs 134.9 ± 122; TFV

derived from TDF, 623.4 ± 248 vs 795.2 ± 162 vs 331.9 ± 51; TFV derived from TAF, 43.06 ± 20 vs 38.8 ± 12 vs 11.47 ± 2.4; and MVC, 627.9 ± 441 vs 1140 ± 514 vs 137.8 ± 49. A statistically significant ($p < 0.05$) increase in EVG, TAF derived TFV LN concentrations was observed when administered through IM and SC routes, whereas, RPV, COBI, TDF derived TFV and MVC concentrations in LN were significantly increased following SC route compared to oral administration.

Conclusions: We conclude that administration of RPV, EVG, COBI, TDF, MVC and TAF through SC route increases their LN concentrations and could provide a potentially useful strategy to enhance ARV concentrations in the LN over the conventional oral route of administration. Future studies may investigate this approach to limit viral replication and reduce viral sanctuaries in the LN, which is a prerequisite for eradication of HIV.

Session 6 and 8: New therapeutic approaches I and II

OP 6.1

Multispecific anti-HIV duoCAR-T cell therapy mediates robust HIV suppression and elimination of HIV-infected cells in humanised mice

K. Anthony-Gonda[1], A. Bardhi[2], A. Ray[2], W. Krueger[1], D. Schneider[1], Z. Zhu[1], R. Orentas[1], D. Dimitrov[3], H. Goldstein[2], B. Dropulic[1]

[1] Lentigen, a Miltenyi Biotec Company, Gaithersburg, USA, [2] Albert Einstein College of Medicine, Bronx, USA, [3] University of Pittsburg, Pittsburg, USA

Background: Adoptive immunotherapy using chimeric antigen receptor (CAR) modified T-cells has shown unprecedented success for the treatment of refractory B-cell malignancies. However, past attempts using 1st generation CD4ζ CAR-T cells for the treatment of HIV were unsuccessful in humans despite long-term persistence of the gene-modified T cells. Here, we aimed to engineer HIV-1-based lentiviral vectors (LV) encoding novel anti-HIV CARs targeting well-conserved sites on the HIV envelope glycoprotein (Env) using a two-molecule architecture termed duoCAR. We hypothesized that anti-HIV duoCARs would significantly improve CAR-T cell potency, breadth, and resistance to HIV infection in contrast to single-molecule CAR (monoCAR) constructs which are similar to the 1st generation CD4ζ CAR.

Methods: To test this hypothesis, we challenged CAR-T cells with donor-matched PBMCs infected with a global panel of replication-competent infectious molecular clones of HIV that encode a *Renilla* luciferase reporter (Env-IMC-LucR). We monitored CAR-mediated suppression of HIV infection by quantifying the LucR activity present in HIV-infected PBMC co-cultures or humanized mice relative to unmodified T cells after 7 days or 30 days of HIV infection, respectively.

Results: We show that transduction with LV encoding multispecific anti-HIV duoCARs conferred primary T cells with the capacity to potently suppress broad strains of Env-IMC-LucR viruses (up to 99%) while simultaneously protecting them from HIV infection. In a humanized NSG model of intrasplenic HIV infection, duoCAR-T cells potently suppressed HIV infection by >97% and eliminated HIV-infected cells significantly better than monoCAR-T cells. Interestingly, mono- and duoCAR-T cells were detected at similar levels in HIV-infected mice despite a difference in their *in vivo* activity. Lastly, multispecific anti-HIV duoCAR-T cell therapy mitigated the loss of human $CD4^+$ T cells in HIV-infected mice.

Conclusions: We conclude that multispecific duoCAR-T cell therapy is broadly-reactive, protective, and superior to monoCAR-T cell therapy. When combined with strategies that target the HIV reservoir, anti-HIV duoCAR-T cell therapy may offer a path towards a functional cure for HIV.

Conflict of interest: I am an employee of Lentigen, a Miltenyi Biotec Company. A patent application has been filed for the work presented in the abstract.

OP 6.2

Location, abundance and persistence of CAR/CXCR5 transduced T cells within lymphoid tissues of SIV-infected rhesus macaques

H. Abdelaal[1], M. Pampusch[1], P. Skinner[1], E. Berger[2]

[1] University of Minnesota, Minneapolis, USA, [2] University of Minnesota, NIH, Bethesda, MD, USA

Background: During chronic HIV-1 and SIV infection, virus replication is concentrated within B cell follicles of secondary lymphoid tissue (SLT), while virus-specific CTL are largely excluded from these sites. This suggests that the inability of virus-specific CTL to fully suppress virus replication may be due to their deficiency in B cell follicles. High levels of follicular SIV-specific CTL inversely correlate with levels of follicular viral replication and with plasma viral loads. These findings support development of a functional cure to target the follicular reservoir of viral replication using T cells co-expressing the B cell follicle homing molecule, (CXCR5) and an SIV-specific chimeric antigen receptor (CD4-MBL-CAR). We hypothesize that CAR/CXCR5 T cells can home to B cell follicles, interact with and kill virus-infected cells resulting in better control of viremia.

Methods: To test these hypotheses, we engineered autologous T cells to express CAR/CXCR5 T cells and infused them into 6 ART-suppressed SIV-infected rhesus macaques at the day of ART interruption. Three untreated ART-suppressed SIV-infected animals served as controls. I used RNAScope *in situ* hybridization combined with immunohistochemistry on paraffin-embedded lymphoid tissue sections to determine the location, abundance, and persistence of the CAR/CXCR5 T cells. I also determined the location and abundance of SIV-infected cells.

Results: Preliminary data show that CAR/CXCR5 T cells successfully home to the B cell follicle of SLT of treated animals, where they show evidence of *in vivo* expansion and direct interaction with virus-infected cells. CAR/CXCR5 T cells were most abundant at 6 days post-treatment (DPT) and lasted for at least 28 DPT. Four of the six treated animals showed better control of viremia relative to the control group and the controlling animals showed the highest level of CAR/CXCR5 T cells in lymph node at 14 DPT.

Conclusions: These Results: demonstrate successful homing of the CAR/CXCR5 T cells to the B cell follicle and direct interaction with virally infected cells *in vivo*. These findings support the CAR/CXCR5 immunotherapy as a functional cure for HIV/SIV infections.

OP 6.3

Combinatorial latency reversal activity of inhibitor of apoptosis antagonists with mechanistically distinct classes of HIV latency reversal agents

Shane D. Falcinelli[1,3], David M. Irlbeck[1,5], Anne-Marie Turner[1], Frances Potjewyd[2], Lindsey I. James[1,2], David M. Margolis[1,3,4], Nancie M. Archin[1,4], Richard M. Dunham[1,5]

[1] UNC HIV Cure Center, [2] Center for Integrative Chemical Biology and Drug Discovery, [3] Deptartment of Microbiology and Immunology, [4] Department of Medicine University of North Carolina, Chapel Hill, NC, USA, [5] HIV Drug Discovery, ViiV Healthcare, Research Triangle Park, NC, USA

Background: Efficacy of HIV latency reversal agents (LRAs) has been limited because of clinical safety issues, and when administered in tolerated regimens, LRAs have not convincingly demonstrated an impact on the functional viral reservoir. As an alternative to the strategy of LRA monotherapy, the use of LRA combinations may (1) induce higher levels of proviral expression (2) reactivate a greater number of latent proviruses, and (3) potentiate latency reversal activity at lower drug exposures for LRAs that have dose-limiting toxicities. We recently reported that Inhibitor of Apoptosis antagonists (IAPa; also known as second mitochondrial-derived activator of caspases mimetics), have potent single agent latency reversal activity *in vivo*. Here, we hypothesized that combination of IAPa with LRAs of distinct mechanistic classes would enhance the magnitude and potency of HIV latency reversal.

Methods: A panel of mechanistically distinct LRAs were screened in 10 point matrix cross titrations with AZD5582, an IAPa, for combinatorial activity in a latent HIV-infected Jurkat T cell line model with a luciferase reporter. LRA combinations that demonstrated Bliss synergy in the Jurkat model were then evaluated for their ability to induce HIV RNA in resting CD4 T cells from aviremic HIV+ participants. The mechanisms of synergistic activity were then further explored using CRISPR and proteolysis targeting chimeras (PROTACs), as well as other molecular biology approaches.

Results: We evaluated combinations of mechanistically distinct LRAs with IAPa, including histone deacetylase (HDAC) inhibitors, protein kinase C agonists, bromodomain inhibitors (BETi), and toll-like receptor

7 agonists using the Bliss Independence model. Combinations of IAPa + HDACi and IAPa + BETi led to synergistic latency reversal in the Jurkat model. Evaluation of IAPa + BETi in resting CD4 T cells demonstrated markedly increased induction of HIV cell-associated gag RNA relative to single agents. Mechanistic investigations of the synergy revealed key insights into the biology underlying latency reversal.

Conclusions: These findings highlight the combinatorial activity between IAPa + HDACi and IAPa + BETi for HIV latency reversal *in vitro*. Evaluation of the ability of these combinations to induce expression of latent virus *in vivo* as part of an approach to reduce the viral reservoir is warranted.

Conflict of interest: David Irlbeck and Richard Dunham are employees of ViiV healthcare.

OP 6.4

α4β7-blockade delays viral rebound in SHIV infected macaques treated with a combination of HIV bNAbs

E. Martinelli

CBR, Population Council, New York, USA

Background: Anti-HIV broadly nAbs (bNAbs) currently in clinical development were selected on the basis of their potent virus-neutralizing activity and breath. However, aside from their direct antiviral effect, bNAbs may favor the development antiviral immunity by engaging the immune system during the period of immunotherapy. Targeting integrin α4β7 with an anti-α4β7 mAb (Rh-α4β7) decreases SIV gut viral loads, leads to changes in immune cell trafficking and impacts immune responses in SIV/SHIV infected macaques. Moreover, Vedolizumab, a humanized mAb FDA-approved against inflammatory bowel disease, with the same antigen-binding region of Rh-α4β7, reduces lymphoid aggregates in the gut of HIV-IBD patients.

Methods: To explore the therapeutic potential of combining anti-HIV bNAbs with α4β7 blockade, 20 macaques with SHIV-SF162P3 plasma viral load (pVL) between 10^3 and 10^5 copies/ml at day 10p.i. were treated with: a) bNAbs-only (n=8; VRC07-523LS at 20mg/kg; PGT128 at 5mg/kg); b) bNAbs + Rh-α4β7 (at 50mg/kg; n=7) or c) left untreated (n=5). One macaque in the bNAbs/Rh-α4β7 group was excluded because of rapid development of ADA against all 3 treatment antibodies.

Results: bNAbs treatment decreased pVL below 200copies/ml in all macaques. 7 out of 8 macaques (87.5%) in the bNAbs-only group rebounded within a median of 3 weeks (95%CI: 2-9). In contrast, 3 out of 6 (50%) macaques in the bNAbs/ Rh-α4β7 group remained below 200 copies/ml until the end of follow up (20weeksp.i.) and the remaining 3 macaques rebounded within a median of 6 weeks after treatment (95%CI: 5-11). Macaques in the bNAbs/Rh-α4β7 group had an increase in memory blood $CD4^+$ T cells and reduced frequency of total B cells, but higher frequency of NK cells in the mesenteric lymph nodes than the other 2 groups. More immunological and virological parameters are being analyzed.

Conclusions: Our pilot study suggests that a combination of 2 bNAbs can effectively decrease pVL for a short period of time and that α4β7 blockade may improve bNAbs-driven therapeutic effect in SHIV-SF162P3 infected macaques

OP 6.5

Replacing daily cART with AAV-expressed eCD4-Ig

M. Gardner, M. Davis-Gardner, M. Farzan

Scripps Research Institute, Jupiter, USA

Background: eCD4-Ig is a broad and potent HIV-1 inhibitor that neutralizes all HIV-1, HIV-2, and SIV isolates tested to date. We have previously shown that a rhesus macaque version of eCD4-Ig (rh-eCD4-Ig) can be expressed in macaques using AAV vectors at concentrations that protect from high-dose SHIV-AD8 and SIVmac239 challenges. Here, we test the hypothesis of whether AAV-expressed rh-eCD4-Ig could maintain SHIV suppression in SHIV-infected macaques that were controlling viremia on combination antiretroviral therapy (cART).

Methods: Ten rhesus macaques were infected with SHIV-AD8. At 12 weeks post infection, macaques were treated with daily cART (a combination of TDF, FTC, and DTG). When viremia was below the limits of detection (<15 viral RNA copies/mL), six macaques were inoculated with AAV8 and AAV1 vectors encoding rh-eCD4-Ig and four macaques were left untreated. At 2-4 weeks after the second AAV inoculation, cART was ended. Viremia and rh-eCD4-Ig concentrations have been measured for the >2 years since cART withdrawal of the six macaques that received treatment.

Results: All 10 macaques had viral rebound 1-4 weeks after cART withdrawal, although macaques treated with AAV-rh-eCD4-Ig had lower rebound viremia. All six treated macaques have been expressing 4-20 ug/mL of rh-eCD4-Ig for nearly two years. Viremia is currently suppressed in these macaques (<100 viral RNA copies/mL) with four macaques at below the limits of detection where control animals ranged from 1400-7000 viral RNA copies/mL before being taken off study. Ongoing efforts now include improving the transgene cassette of the AAV vectors to increase rh-eCD4-Ig expression that can limit viral rebound after cART withdrawal and suppress SIVmac239 infection.

Conclusions: AAV-expressed rh-eCD4-Ig can suppress SHIV-AD8 viremia over a year after cART withdrawal. With further optimizations to increase the concentrations of expressed rh-eCD4-Ig, this therapy may become a replacement to daily cART.

Conflict of interest: MG and MF and co-founders and shareholders of Emmune, Inc., a company that is licensing eCD4-Ig techonology for the purpose of developing eCD4-Ig for clinical trials.

OP 8.1

The human IL-15 superagonist N803 does not reverse latency in ART-suppressed, SHIV-infected macaques

G. Webb[1], J. Berrocal[2], K. Busman-Sahay[1], S. Abdulhaqq[1], J. Smedley[1], J. Safrit[3], J. Estes[1], P. Skinner[2], J. Sacha[1]

[1] Oregon Health and Science University, Portland, USA, [2] University of Minnesota, St. Paul, USA, [3] NantKWest, Culver City, USA

Background: There is an urgent need for alternate approaches to clear the HIV reservoir. A hurdle to clearing the reservoir is the exclusion of CD8+ T cells from B-cell follicles, a site that harbors latently-infected CD4+ T cells. IL-15 is a key cytokine for homeostatic maintenance, proliferation, and expansion of memory CD4+ T cells, the primary HIV cellular reservoir. Here, we explored the human IL-15 superagonist complex, N803, as an immunostimulatory molecule and latency-reversal agent (LRA) in ART-suppressed, SHIV-infected rhesus macaques (RM).

Methods: Fully ART-suppressed, SHIVsf162p3-infected RMs were treated with 4 subcutaneous doses of 100mg/kg N803, administered biweekly. Plasma viral load monitoring and RNA in situ hybridization of lymph node (LN) sections were used to measure episodes of virus reactivation during N803 treatments. Animals were subsequently assessed for intrafollicular migration of SHIV-specific CD8+ T cells via in situ immunofluorescence staining of LNs with MHC-class-I tetramers as well as NK cell migration via immunofluorescence staining. Cell-associated viral DNA was assessed by qPCR.

Results: N803 activated NK cells and memory CD8+ T cells, triggering proliferation and homing to secondary lymphoid tissues. In situ MHC-class-I tetramer staining and immunofluorescence staining revealed trafficking of these effectors into B-cell follicles. We found no difference in the number of plasma virema episodes between groups and no change in the size of the latent reservoir, yet after ART discontinuation the N803 group displayed a trend towards delayed rebound kinetics of 1.5 weeks.

Conclusions: IL-15 superagonist, N803, triggers massive proliferation of NK cells and CD8+ T cells resulting in NK and CD8+ T cell accumulation in LNs, specifically in B-cell follicles harboring latently-infected

CD4+ TFH cells. These Results: suggest that while N803 is able to localize effector cells to the sites of the latent reservoir, N803 itself has no appreciable direct ability to reactivate latent virus. In order to fully unlock the potential of N803 as an anti-HIV therapeutic, it must be paired with a compound capable of potentiating reactivation of latent virus.

OP 8.2

How long is long-term? Delivery of anti-HIV antibodies using AAV vector

J. Martinez-Navio[1], R. Desrosiers[1], S. Fuchs[1], D. Mendes[1], E. Rakasz[2], G. Gao[3], J. Lifson[4]

[1] University of Miami, Miami, USA, [2] Wisconsin National Primate Research Center UW, Madison, USA, [3] Gene Therapy Center UMass, Worcester, USA, [4] Frederick National Laboratory for Cancer Research, Frederick, USA

Background: Long-term delivery of monoclonal antibodies using adeno-associated virus (AAV) holds promise for both, prevention and treatment of HIV infection. We have previously reported two shining examples, one for each of these two applications using the AAV approach. In terms of prevention, we reported monkey 84-05, which after receiving one single administration of AAV vector coding for anti-SIV antibody 5L7, achieved high levels of AAV-delivered 5L7 which conferred sterile protection to six successive challenges with SIVmac239, including a final 10x dose (PLoS Pathog 2015). In terms of treatment, we recently reported monkey rh2438 in which a single administration of AAVs encoding a combination of potent and broadly neutralizing antibodies during the chronic phase of infection resulted in an abrupt decline in plasma viremia which remained below the limit of detection for over a 3-year period (Immunity 2019).

Methods: Indian-origin rhesus macaques received recombinant AAV vectors expressing IgG1 versions of the selected antibodies. When a therapy approach was used, monkeys were infected with SHIV-AD8 months before AAV administration. Antibody levels were measured by ELISA.

Results: Here we report that monkey 84-05 has successfully maintained 240-350 μg/ml of anti-SIV antibody 5L7 for over 6 years. Approximately 3% of the circulating IgG in this monkey is this one monoclonal antibody. Ultrasensitive cell-associated viral DNA/RNA tests, and ELISAs against overlapping peptides spanning the whole SIVmac239 envelope sequence and against p27 and gp41 proteins have ruled out a potential low-level infection not detected by regular viral loads. We conclude that macaque 84-05 was effectively protected and remained uninfected. We also report that monkey rh2438 continues to be suppressed (4 years and counting) and expressing high levels of AAV-delivered antibodies. Additionally, we have two other macaques, r14121 and r14097, which have had suppressed viral loads for the last 9 months. Both monkeys show substantial antibody levels of 3 of the 4 antibodies that they received.

Conclusions: Our data show that durable, continuous antibody expression can be achieved after one single administration of AAV and support the potential for a) lifelong protection against HIV acquisition and b) lifelong suppression of viral loads, both from a single vector administration.

OP 8.3

Intensification of ART with ABX464 decreases the total HIV reservoir and HIV transcription initiation in CD4 T cells from HIV-infected ART-suppressed individuals

S. Moron-Lopez[1], S. Bernal[2], J.M. Steens[3], J.K. Wong[4], J. Martinez-Picado[2], S.A. Yukl[4]

[1] University of California San Francisco, San Francisco, USA, [2] IrsiCaixa AIDS Research Institute, Badalona, Spain, [3] ABIVAX, Paris, France, [4] San Francisco VA Medical Center, San Francisco, USA

Background: Antiretroviral treatment (ART) intensification and disruption of latent HIV infection have been suggested as strategies to eradicate HIV-1. ABX464 is a novel antiviral that binds to the cap binding complex, interfering with splicing and Rev-mediated export of HIV RNA. ABX464 has been shown to inhibit HIV RNA biogenesis in vitro and delayed viral rebound in a humanized mouse model. We investigated the effect of ABX464 on the HIV transcription profile and total and intact HIV DNA in CD4+ T cells from ART-suppressed participants enrolled in the ABIVAX-005 clinical trial (NCT02990325).

Methods: Eleven participants on suppressive ART were treated daily with 150mg of ABX464 for 4 weeks. Peripheral CD4+ T cells from nine study participants were available for HIV transcription profile and reservoir size analysis. Total HIV DNA, intact HIV DNA (IPDA), and Read-through, total/initiated, 5′elongated, unspliced, polyadenylated and multiply-spliced Tat-Rev HIV transcripts were quantified at weeks 0, 4 and 8 using ddPCR.

Results: We observed a significant decrease in the total HIV DNA (p=0.008, median fold-change=0.8) and a lower median level of intact HIV DNA (p=n.s., median fold-change=0.8) after ABX464 treatment (wk0vs.4). However, intact HIV DNA increased significantly (p=0.008, fold-change=1.6) after ABX464 discontinuation (wk4 vs. wk8). After 4 weeks of ABX464 treatment, we observed a decrease in total initiated HIV RNA per million CD4+ T cells and per provirus (HIV RNA/HIV DNA) (p=0.05, median fold-change=0.7; p=0.004, median fold-change=0.5, respectively), a trend towards a decrease in the 5′elongated HIV RNA per provirus (p=0.07, median fold-change=0.5), and a lower median level of unspliced HIV RNA (p=n.s., median fold-change=0.6), but no decrease in polyadenylated or multiply-spliced HIV RNA. However, 5′elongated HIV RNA per million CD4+ T cells increased significantly (p=0.04, fold-change=1.4) after ABX464 discontinuation (wk4vs.8).

Conclusions: In this substudy, ABX464 had a dual effect of decreasing total HIV DNA (and possibly intact proviruses) and decreasing the amount of HIV transcription per provirus, although these changes were reversed after drug discontinuation. Our data suggest that ABX464 acts as an ART intensifier in vivo. To further characterize its specific mechanism of inhibiting HIV transcription, long-term administration of ABX464 in a larger cohort should be studied.

Conflict of interest: Jean-Marc Steen is employee of AbiVax, JMP received grant support and consultancy fees from AbiVax

OP 8.4

HIV persistence despite reservoir decay during combinatorial immunotherapy including therapeutic conserved elements (CE) DNA vaccination, αPD-1 therapy, GS-986 TLR7-agonism, and CCR5 gene-edited CD4 T cell infusion in rhesus macaques

S. Dross[1], C. Peterson[2], M. O'Connor[1], H. Tunggal[1], J. Li[1], K. Jerome[2], H.P. Kiem[2], B. Felber[3], J. Mullins[1], D. Fuller[1]

[1] Department of Microbiology, University of Washington, Seattle, USA, [2] Fred Hutchinson Cancer Research Center, Seattle, USA, [3] Human Retrovirus Pathogenesis Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute, Frederick, USA

Background: HIV persistence is aided by immune evasion and exhaustion. In a SHIV+ non-human primate model, we tested a carefully timed combination of immunotherapies during cART. We hypothesized a combination of therapeutic DNA vaccination targeting viral conserved elements (CE), αPD-1 reversing immune exhaustion, GS-986 TLR7-agonism activating latently infected cells, and CCR5-gene-protection of CD4+ T-cells would decrease or eliminate viral persistence.

Methods: Twenty-four SHIV-1157ipd3N4-infected rhesus macaques began cART 7 weeks post-infection (wpi). Combinatorial immunotherapy began 33wpi: 8 animals received all immunotherapies (CE/αPD1/GS986/ΔCCR5), 8 received DNA vaccination and CCR5-editing (CE/ΔCCR5), and 8 received cART alone (controls). Vaccine responses were measured 28, 56, 75 and 81wpi in the blood (PBMC) and mesenteric lymph nodes (MLN) by intracellular staining. Viral reservoir decay in apheresed PBMC was measured

by SHIV-QVOA (Quantitative Viral Outgrowth Assay). Intervention efficacy was measured by viral load analyses during analytic treatment interruption (ATI).

Results: Vaccine responses peaked at 56wpi and waned by 75wpi despite the 5th vaccination at 73wpi. T-cell responses in the CE/αPD1/GS986/ΔCCR5 group were elevated 56wpi in PBMC but not MLN, suggesting that αPD-1 may enhance vaccine responsiveness but not permeate the gut-associated lymphoid tissues (GALT). Significant reservoir decay ($p<0.01$) during interventions was found in the peripheral blood by QVOA in the CE/αPD1/GS986/ΔCCR5 group, however during ATI the time-to-viral rebound and viral loads were similar in all groups. Polyfunctional T-cell CE responses in the MLN during ATI correlated inversely with ATI viral burden suggesting GALT CE T-cell responses are important for viral control.

Conclusions: CE DNA vaccination of SHIV-infected, cART-suppressed macaques elicited CE T-cell responses with αPD-1 improving PBMC, but not MLN, responses. These polyfunctional CE responses in the GALT were associated with viral control during ATI. QVOA-measured reservoir decay was detected in CE/αPD1/GS986/ΔCCR5 animals. QVOA analyses of CE/ΔCCR5 and control animals are ongoing as are analyses of exhaustion reversal in PBMC vs. GALT in all animals. Our collective findings imply optimization of our immunotherapies to enhance GALT trafficking, exhaustion reversal and vaccine response durability is crucial to reduce or eliminate viral persistence during therapy.

OP 8.5

PD-1 blockade boost vaccine-induced anti-HIV responses in the absence of HIV reactivation

J.G. Prado[1], M. Marin Lopez[1], E. Jimenez-Moyano[1], D. Ouchi[1], O. Blanch-Lombarte[1], D. Gorman[2], T. Hanke[3], C. Brander[1], B. Howell[4], B. Mothe[1]

[1] IrisiCaixa, Badalona, Spain, [2] Merck & Co. Inc., Palo Alto, California, USA, [3] Jenner Institute Nuffield Department of Medicine, University of Oxford, Oxford, UK, [4] Department of Infectious Disease, Merck & Co. Inc., Kenilworth, NJ, USA

Background: To attain the control or elimination of HIV-1 infection, it is critical to delineate new therapeutic approaches capable of boosting HIV-1-specific $CD8^+$ responses. Immune interventions, including therapeutic vaccines or immune checkpoint inhibitors (ICIs), have been postulated to achieve this goal. However, the potency of combining both immune interventions has not been tested. Here, we assessed ex-vivo the impact of ICI on vaccine-induced HIV-1 $CD8^+$ responses and viral reactivation using samples from a vaccine trial conducted in early-treated HIV-1 infected individuals.

Methods: We selected PBMCs of individuals from the BCN01-NCT01712425 trial receiving early treatment and ChAdV63.HIVconsv/MVA.HIVconsv prime-boost regimen (Etvac;n=12). For comparison, we selected PBMCs from early treated (Et;n=13) and chronically treated individuals (Chro;n=11). PBMCs were CFSE-stained and stimulated with an HIV-1 peptide pool in the presence of anti-PD-1, anti-TIM-3, anti-PD-1+TIM-3 or isotype antibodies. After seven days, we quantified the frequency of CFSE-, $IFN\gamma^+$ and $HLA\text{-}DR^+/CD38^+$ HIV-1-specific $CD8^+$ T cells by flow cytometry. Additionally, we measured a panel of 17 human cytokines and performed ultrasensitive p24 determination in the culture supernatants.

Results: Anti-PD-1 boosted Etvac response in terms of proliferation ($p=0.004$), IFNγ production ($p=0.04$), and $HLADR^+/CD38^+$ expression ($p=0.004$). These Results: were consistent for anti-PD-1+TIM-3 in the absence of response to anti-TIM-3. However, ICI in Et did not alter significantly the frequency of HIV-1-specific $CD8^+$ responses while Chro showed an increase in the frequency of HIV-1-specific $CD8^+$ responses upon PD-1 or PD-1+TIM-3 inhibition. Etvac cytokine profiling revealed a signature of IFNγ, sFasL, GM-CSF, sCD137, IL-13, Granzyme-A, Granzyme-B, MIP-1β and Perforin secretion in response to anti-PD-1 that differed in Chro by the lack of IL-13, MIP-1β and Perforin but the presence of IL-10 and IL-2. Additionally, in our experimental setting ICI did not induce a reactivation of HIV reservoir based on p24 ultrasensitive detection.

Conclusions: Our data demonstrate a significant increase in the magnitude of vaccine-induced HIV-1-specific $CD8^+$ responses by ICIs linked to a particular cytokine profiling in Etvac. Thus, we propose the combinatorial use of ICI and therapeutic vaccines to boost vaccine-induced anti-HIV $CD8^+$ responses as new therapeutic approach. However, lack of HIV reactivation by ICI warrants further investigation in the context of cure strategies.

Conflict of interest: DG and BH are employed by Merck. J.G.P have received grants from MSD (Merck). All remaining authors have declared no conflicts of interest.

OP 8.6

Post-therapy viral set-point abatement following combined antiproliferative and immune-boosting interventions: results from a randomised clinical trial

R. Sobhie Diaz[1], L.B. Giron[2], J. Galinskas[1], J. Hunter[1], M. Janini[1], I.L. Shytaj[3], R. Cauda[4], M.C. Sucupira[5], J. Maricato[5], A. Savarino[6]

[1] Federal University of Sao Paulo, Infectious Diseases Department, São Paulo, Brazil, [2] Wistar Institute, Philadelphia, USA, [3] Heidelberg University Hospital, Department of Infectious Diseases, Heidelberg, Germany, [4] Institute of Infectious Diseases, Gemelli Hospital, Catholic University of Sacred Heart, Rome, Italy, [5] Federal University of Sao Paulo, Infectious Diseases Department, Sao Paulo, Brazil, [6] Department of Infectious Diseases, Italian Institute of Health, Rome, Italy

Background: Men living with HIV/AIDS with viral loads (VL) suppressed by antiretroviral therapy (ART) for >2 years (CD4 counts >350 cells/μL) were randomized to the open-label SPARC-7 TRIAL interventions (Federal University of Sao Paulo, Brazil). The following treatments were conducted in parallel for 48 weeks: G1 baseline ART continuation, G2) ART + dolutegravir/maraviroc intensification, G3) ART + dolutegravir/maraviroc + nicotinamide, G4) ART + dolutegravir/maraviroc + nicotinamide + auranofin, G5) ART + dolutegravir + personalized dendritic cell vaccine based on HLA-matched Gag epitopes and designed by the study investigators, G6 baseline ART + dolutegravir + nicotinamide + auranofin + vaccine as above. Auranofin was used for the first 24 weeks. The vaccine was administered at the end of the 48 weeks (3 doses with 2 week intervals.

Methods: We tested whether the antiproliferative approach using auranofin/nicotinamide and immunity boosting might impact on viral DNA and on viral load (VL after analytical treatment interruption (ATI). Viral DNA was quantified by two independent qPCR techniques and VL was measured using the Ability platform (detection limit: 40 copies/ml).

Results: Five individuals were randomized to each group. There were no severe adverse events. 28/30 individuals remained treatment compliant. Viral DNA decrease to undetectable levels was observed only in G6 at 48 weeks (per-protocol analysis: $p=0.022$; Odds ratio: 9.75, 95%CL: 1.1-72.39). Following ethical approval, ATI was conducted in 25 individuals who gave informed consent.VL rebound in G6 was significantly lower than in the other groups (Figure 1A). To exclude contribution of pre-therapy VLs to post-treatment VL control, pre- and post-ART VL set points of G6 were compared, resulting in a significant difference (Figure 1B). Of note, two ofthe four therapy-compliant individuals displayed a set point <1000 viralRNA copies/mL and stable CD4 counts for 5 months during ATI. These individuals had also displayed undetectable viral DNA before ATI.

Conclusions: The present study provides proof of concept that the antiproliferative approach in combination with ART intensification and immunity boosting impacts on viral DNA and improves post-treatment control of viral load. Another therapeutic cycle in these subjects is planned to evaluate whether sustained remission without ART may be obtained.

Conflict of interest: RSD, ILS, AS have requested patent rights on some of the study interventions.

OP 8.7

B cell depletion alone or in combination with IL-15 or PD-1 blockade facilitates enhanced control of virus replication in SIV-infected rhesus macaques

L.J. Picker[1], Y. Fukazawa[1], H. Behrns[1], B.E. Randall[1], B. Varco-Merth[1], H. Park[1], B.K. Felber[2], G.N. Pavlakis[2], J.D. Lifson[3], A.A. Okoye[1]

[1] Vaccine and Gene Therapy Institute and Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, Or, USA, [2] Center for Cancer Research, National Cancer Institute, Frederick, MD, USA, [3] AIDS and Cancer Virus Program, Frederick National Laboratory, Frederick, MD, USA

Background: We have previously reported on the exquisite restriction of replication-competent virus to CD4+ follicular helper T cells resident within B cell follicles of secondary lymphoid tissues of rhesus macaques (RM) with elite control (EC) of pathogenic SIV infection, suggesting that the highly effective anti-viral CD8+ T cell responses in these RM are able to almost completely clear and/or suppress productive SIV infection in extra-follicular T cell zones but not within B cell follicles. Here we evaluated whether B cell depletion (to disrupt B cell follicles) alone or in combination with IL-15 or PD-1 blockade (to improve the functional activity of SIV-specific T cells), can enhance control of virus replication.

Methods: A cohort of 7 SIVmac239-infected EC RM (plasma viral loads [pvl] <1,000 RNA copies/ml) received up to 3 intravenous (IV) doses of a depleting anti-CD20 antibody (Ab) at 50mg/kg. A second cohort of 7 EC RM received 4 doses of anti-CD20 in combination with 2 doses of a rhesus anti-PD1 Ab (IV at 3 mg/kg). A third cohort of 6 EC RM received subcutaneous injections of a rhesus heterodimeric IL-15 (hetIL-15) alone or in combination with anti-CD20 Ab. Pvl (RNA copies/ml) were quantified by qRT-PCR and lymphocyte population dynamics assessed by flow cytometry and immunohistochemistry.

Results: Anti-CD20 Ab alone or in combination with PD-1 blockade resulted in rapid 1-2 log reductions in the already low pvl in these EC RM, suggesting disruption of follicles by B cell depletion improves overall viral control. However, while anti-CD20 was effective at depleting B cells in blood and bone marrow, depletion in peripheral lymph nodes (LN) was often incomplete. Strikingly, RM treated with anti-CD20 in combination with hetIL-15 also showed 1-2 log reductions in pvl in addition to more effective B cell depletion in LN. In all cohorts, viral control was transient, as pvl generally returned to baseline levels in concert with recovery in B cell counts.

Conclusions: Overall, these data suggest that B cell follicles constitute a significant barrier to the utility of virus-specific CD8+ T cells to control virus replication and facilitate durable virus remission after ART cessation.

Conflict of interest: George N. Pavlakis and Barbara K. Felber are inventors on hetIL-15 patents owned by the USA Government.

PP 6.8.1

Targeting lncRNA SAF to induce apoptosis in HIV-1 infected macrophages

S. Boliar[1], D.W. Gludish[1], K.C. Jambo[2], H.C. Mwandumba[2], D.G. Russell[1]

[1] Cornell University, Ithaca, USA, [2] University of Malawi, Blantyre, Malawi

Background: Long non-coding RNAs (lncRNA) are emerging as pivotal players in regulation of a multitude of biological pathways and therefore are appealing as important targets for modulation in treatment of various diseases including HIV-1. The role of lncRNAs in HIV-1 pathogenesis is under-studied, particularly in macrophages, which are notably resistant to the virus-induced cell death and constitute a long-lived cellular reservoir of HIV-1. Here we have examined and identified the role of a lncRNA in regulation of apoptosis of HIV-1 infected cells and effects of modulation of the lncRNA on the cellular fate of the virus-infected macrophages.

Methods: Human monocyte-derived macrophages (HMDM) were infected with VSV-G pseudotyped NL4-3 HIV-1 virus expressing the R5-tropic BaL Env. Alveolar macrophages (AM) were obtained from lung of HIV-1 infected individuals by bronchoalveolar lavage. Expression of ninety well-characterized lncRNAs in virus-infected, bystander and virus non-exposed HMDMs were determined using a quantitative RT-PCR array. Additionally, siRNA was used for targeted down-regulation of specific lncRNA and its effect on apoptosis of HIV-1 infected HMDMs was determined by flow cytometric detection of active caspase-3 in the cells.

Results: HMDMs supported productive HIV-1 infection without viral-induced cytopathic effect. A divergent pattern of lncRNAs expression was observed between virus infected and bystander HMDMs. In particular, expression of the anti-apoptotic lncRNA SAF (FAS-AS1) was found to be significantly up-regulated in HIV-1 infected HMDMs in comparison to bystander and non-exposed cells. Similar increase in SAF expression was also observed in HIV-1-positive lung AMs. Down-regulation of SAF lncRNA with siRNA led to a significant increase in active caspase-3 levels in HIV-1 infected HMDMs. This induction of apoptosis occurred specifically in HIV-1 infected HMDMs but not in bystander or virus non-exposed cells. The targeted cell death of virus-infected cells resulted in a significant reduction in viral load in the HIV-1 infected macrophage culture.

Conclusions: The lncRNA SAF plays a key role in cell survival of HIV-1 infected macrophages and siRNA-mediated modulation of this lncRNA offers a potential new therapeutic approach to specifically eliminate virus-infected macrophage reservoirs.

PP 6.8.2

Blocking TIM-3 reinvigorates exhausted CD8 T cells with no impact on NK cell function in ART-treated HIV-infected patients

C. Gutiérrez, M. Sanz, N. Madrid-Elena, S. Serrano-Villar, S. Moreno

Infectious Diseases Department, Ramón y Cajal Hospital, Madrid, Spain

Background: TIM-3 is a large transmembrane inhibitory receptor that is expressed in multiple cells of the immune system, including T-CD8+ cells and NK cells. TIM-3 binds to Galectin-9, which has been described to be a potent mediator of HIV transcription and reactivation. We analyze the effect of TIM-3 blockade on the specific HIV-1 CTL response of T-CD8+ cells and NK cells from HIV-infected-patients.

Methods: Peripheral blood (200 mL) for the isolation of primary CD4+, CD8+ and NK cells was obtained from 10 ART-treated, HIV-1-infected donors. T-CD4+ cells were cocultured with T-CD8+ cells and NK cells in a 1:1 ratio. An infection with a BaL virus was carried out. A specific antibody against TIM-3 was added to the coculture. The impact of TIM-3 blockade on the HIV-suppressive capacity of T-CD8+ cells was determined by measuring p24 levels in the supernatants at d7 and d10. Cocultures without adding anti-TIM-3 antibody were used as controls. To evaluate the impact of the natural ligand of TIM-3, p24 levels were compared in cocultures with or without the addition of exogenous Galectin 9.

Results: All the patients had HIV RNA<50 copies/mL, mean CD4+ 661 cells/mm3, mean CD8+ 920 cells/mm3 and mean nadir CD4+ 327 cells/mm3. The average time with ART was 88 months. The HIV-suppressive capacity of T-CD8+ cells was poor (mean p24 decrease, 0.9log), but it was significantly improved after TIM-3 blockade (mean 2.4log), mean difference 1.5log (IQR, [0.4–2.20], p=0.007). Of note, the impact of TIM-3 blockade was similar in the presence of NK cells in the coculture (mean difference with and without blockade, 1.15log [0.49-1.69], p=0.011), after the addition of Galectin-9 (mean difference, 1.25log [0.77-1.42], p=0.012) or with the addition of both NK and galectin (mean difference 1.37log [0.080-1.87], p=0.04) (Figure).

Conclusions: The blockade of TIM-3 restores the CTL response of the CD8+ cells of ART-treated HIV-infected patients. This same blockage in NK cells had no negative effects on the overall CTL response. Since Galectin-9 does not have any impact on the response, a combination of Galectin-9/TIM-3 could be evaluated as an effective strategy in HIV-eradication.

PP 6.8.3

Isolation of monoclonal antibodies targeting HLA-E/HIV-1 and SIV peptide complexes

D. Li[1], S. Brackenridge[2], M. Tuyishime[3], D. Cain[1], Z. Mu[1], B. Mattia[1], G. Ferrari[3], G. Geraldine[2], A. Mcmichael[2], B. Haynes[1]

[1] Duke Human Vaccine Institute, Duke University, Durham, USA, [2] Nuffield Department of Medicine, University of Oxford, Oxford, UK, [3] Department of Surgery, Duke University, Durham, USA

Background: Human leukocyte antigen E (HLA-E) is a non-classical class Ib molecule with limited polymorphism. In the setting of vaccination, protection against simian immunodeficiency virus (SIV) was correlated with rhesus HLA - Mamu E-restricted presentation of SIV gag peptides to CD8+ T cells. Additionally, human HLA-E is also capable of binding and presenting HIV-1 gag peptides. Our goal is to develop monoclonal antibodies (mAbs)specificforthe HLA-E/HIVgag or HLA-E/SIVgag peptide complexes as well as the endogenous VL9 leader sequence peptide-E complex that is a natural ligand for the NK inhibitory receptors NKG2A/CD94. These mAbs could be critical tools to regulate HLA-E-restricted CD8+ T cell responses, and may be capable of recognizing HIV-1 infected T cells and enable development of antibody-dependent cellular cytotoxicity (ADCC)- or cytotoxic T lymphocyte (CTL)- based HIV-1 therapy strategies.

Methods: HumanHLA-B27/β2Mtransgenicmicewereimmunizedwith cysteine-trap-stabilized HLA-E/HIVgag(HLA-E/RL9HIV),HLA-E/SIVgag(HLA-E/RL9SIV) or HLA-E/VL9 peptides. MAbs were isolated either by spleen cell fusion/hybridomas or by single cell sorting/PCR. The mAb candidates were screenedforspecific binding to cell surface expressing HLA-E/RL9HIV, HLA-E/RL9SIV or HLA-E/VL9 byflow cytometry.Human mAbs were isolated from human donors using single B cultures or cell sorting/PCR strategy. MAb variable region genes were engineered into human IgG backbones and tested for ELISA, SPR and cell surface staining.

Results: Two mAbs specific for HLA-E/RL9HIV and/or HLA-E/RL9SIV (19D6, 12D3) as well as one mAb reactive with HLA-EBL9 (3H4) were isolated. By single B cell sorting/PCR approach, wehave isolated one additional mAb from immunized miceand six additional mAbs from HIV-1 negative human specific for bothHLA-E/RL9HIV and HLA-E/RL9SIV. The specificities of these antibodies were validated by cell surface staining. However, none of these mAbs can bound to soluble HLA-E/RL9HIV or RL9SIV in ELISA or SPR, suggesting that the recognition of this group of mAbs require cell surface-bound conformation. The 3H4 HLA-EVL9 antibody bound to HLA-EVL9+ target cells and increased their killing when added to NK-target cocultures.

Conclusions: We have isolated a panel of mAbs that differentially bound to HLA-E/RL9HIV, HLA-E/RL9SIV or HLA-EVL9. This strategy may generate useful reagents that can either target HIV-infected CD4 T cells or modulate NK cell activity.

PP 6.8.4

Improved killing of HIV-infected cells by a combination of three antibodies: implications for clearing persistent infection

M. Tuyishime[1], C. Garrido[2], S. Jha[1], D. Mielke[1], M. Moeser[3], B. Haynes[4], S. Joseph[3], D. Margolis[5], G. Ferrari[1]

[1] Department of Surgery, Duke University Medical Center, Durham, USA, [2] UNC HIV Cure Center and Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, USA, [3] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, USA, [4] Duke Human Vaccine Institute, Department of Medicine, Duke University Medical Center, Durham, USA, [5] Departments of Microbiology and Immunology, and Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, USA

Background: Correlation of Antibody-Dependent Cellular Cytotoxicity (ADCC) responses with protection from HIV-1 infection provides the rationale to leverage them for treatment purposes. We evaluated ADCC mediated by monoclonal antibodies (mAbs) in different combinations to identify those effective against latent reservoir viruses (LRVs) isolated from resting CD4+ T cell reservoir of ART-suppressed HIV+ donors and Latency Reversing Agents (LRA)-exposed latently infected cells.

Methods: HIV-1 envelope-specific CD4-binding site CH557, C1/C2 A32, V1/V2 PG9, V3 PGT121, gp120-gp41 interface PGT151 and MPER DH511.2_K3 mAbs were tested at a concentration ≤1μg/mL for their ability to mediate ADCC individually and in combinations. As target cells, we used: 1) primary CD4+ T cells infected *in vitro* with a panel of 10 clade B LRVs using an Infected Cell Elimination assay; 2) ART-suppressed HIV+ donor cells exposed to LRAs *ex vivo* with a modified viral outgrowth assay (Latent Clearance Assay) in presence of autologous primary NK cells.

Results: We observed that within 2 hrs of incubation at ≤1μg/mL, specific killing was increased ($p<0.005$) by 3 mAbs compared to single or paired mAbs. Addition of more than 3 mAbs did not further increase specific killing. A32/DH511.2_K3/PGT121 was the best combination with a mean of 33.1% ADCC activity. Three LRVs were resistance to neutralization by PGT121, one of which (P800) was also resistant to PG9 and DH511.2_K3. The resistance was caused by the escape mutations within HIV-1 Env of these LRVs. These Results: were confirmed by the loss of the binding of PGT121 and/or DH511.2_K3 to cells infected with these LRVs. After 24 hrs of incubation, we observed remarkably increased ADCC activity against 4 LRVs tested with the A32/DH511.2_K3/PGT121 combination, and demonstrated elimination of cells infected with 3-bNAbs-resistant P800 LRV. Further, the A32/DH511.2_K3/PGT121 combination efficiently cleared *ex vivo* latently infected LRA-treated resting CD4+T cells from P800 donor by autologous NK cells during a 25-day culture.

Conclusions: MAbs combinations targeted the diverse HIV-1 Envs on the surface of infected cells, upon reactivation of the latent infection, more efficiently than individual mAbs. The Results: strongly indicate the importance of mAb combinations to achieve the broadest activity against HIV-1 infected cells in future cure strategies.

PP 6.8.5

Maraviroc reactivates HIV with a potency similar to that of other latency reversing drugs without inducing toxicity in CD8 T cells

S. Moreno, M. López Huertas, L. Jiménez Tormo, N. Madrid Elena, C. Gutierrez, M.J. Vivancos, L. Luna

Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain

Background: Efforts to achieve a functional cure for HIV infection rely partially on immunomodulatory strategies to enhance cytotoxic functions. Implementation of these actions requires a coordinated activation of the viral transcription in latently infected cells so that the reservoir became accessible to cytotoxic cells. As no latency reversing agent (LRA) has been shown to be completely effective, finding new combinations is important. Recent data have shown that maraviroc (MVC) is a new LRA. We have explored how the combination of MVC with other LRAs affects HIV replication andthe cytotoxic function of CD8 T cells.

Methods: Tested LRAs: MVC (5μM, 0.05μM), panobinostat (30nM), disulfiram (500nM), and romidepsin (40nM). Cell viability and proliferation were measured through the ATP and ki67 levels. HIV infections were performed with X4-tropic-NL4.3 or R5-tropic-JR-SCF strains using in vitro latency models based on treating resting (r)CD4 T cells with CCL19 or IL-7. p24/gag levels were measured after LRA

stimulation for 24h or 72h. The viral inhibition assay was performed with cells from patients on antiretroviral treatment (ART) using R5 HIV (ratio CD4:CD81:1). Nevirapine was added at the same time than LRAs and stimulation lasted for 6 days.

Results: In CCL19-treated rCD4 T cells, MVC reactivated X4 HIV with a potency similar to other LRAs (Fig.A). These combinations did not affect viability neither proliferation (Fig.1B). We did not detect an additive or synergistic effect of the combinations on viral reactivation. The combination of MVC with panobinostat or disulfiram maintained the effect of both drugs. These Results: were confirmed using R5 HIV and IL-7-model. Finally, MVC did not alter the viability of CD8 T cells from patients on ART after 16 days (Fig.1C), although it was reduced after stimulation with disulfiram or panobinostat from 2 days onwards. The ability of CD8 T cells to block HIV replication was not changed in CD4 T cells treated with MVC alone or combined with panobinostat or disulfiram (Fig.1D).

Conclusions: MVC is a LRA as potent as disulfiram, romidepsin or panobinostat. In contrast to these LRAs, MVC did not affect viability of CD8 T cells from ART-treated patients.

PP 6.8.6

Modulated production of endogenous anti-HIV broadly neutralising antibodies

Y. Gao[1], D. Patel[2], C. Ding[1], Y. Ma[2], W. Li[1], R. Dekoter[2]

[1] First Affiliated Hospital, University of Science and Technology of China, Hefei, China, [2] Department of Microbiology and Immunology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Canada

Background: Although many anti-HIV broadly neutralizing antibodies (bNAbs) have been identified, progress has been very limited in developing specific HIV immunogens that can elicit a bNAb response. The experiments using bNAbs for passive immunization have shown that it can provide protection. However, due to the short half-life of the antibody, repeated injections are required, and thus large-scale applications are limited. The use of gene transfer to confer host anti-HIV bNAb expression is becoming a very attractive strategy. The present study aimed to use antigen-specific B cells for bNAb gene transfer to develop a modulated expression strategy of bNAb to overcome the problem of insufficient expression of bNAbs in vivo.

Methods: We constructed a pseudoviral particle expressing anti-HIV bNAb (VRC01) using a mouse retroviral vector (MIGR) and validated in vitro, and then transduced hemagglutinin (HA)-specific B cells isolated from influenza vaccine pre-immunized mice. The transduced B cells were transfused back into the flu vaccine pre-immunized recipient mice. The recipient mice were again vaccinated with influenza at day 1 and every other week for totally four times. The mouse sera were finally detected by ELISA to investigate the VRC01 expression level and its function.

Results: The MIG-VRC01 retroviral vector expressing VRC01 was successfully constructed, and the corresponding pseudovirus particles were prepared, and both the anti-p24 hybridoma cells and primary B cells were successfully transduced and produced VRC01 antibody. In vivo modulation experiments in mouse showed that influenza vaccine could successfully stimulate VRC01-transduced HA-specific B cells after transfusion, and the VRC01 expression level in recipient mice increased more than 100-fold after receiving the third immunization and could effectively neutralize different HIV strains.

Conclusions: This study shows that anti-HIV bNAb (VRC01)-transduced HA-specific B cells can be activated by influenza vaccine immunization, suggesting that after a series of experimental procedures such as in vitro culture, stimulation, and transduction, the primary antigen-specific B cells are still able to respond in vivo to stimulation by specific antigen. These B cells can furthermore proliferate and produce large amounts of bNAbs. This study provides a new strategy for anti-HIV bNAb applications that may be used to prevent and treat HIV infection.

PP 6.8.7

CD4 T cells from patients with chronic myeloid leukemia are resistant to HIV-1 proviral integration and transcription after prolonged withdrawal of treatment with tyrosine kinase inhibitors

Lorena Vigón[1], Sara Rodríguez-Mora[1], Elena Mateos[1], Valentín García[2], Juan Ambrosioni[3], Nuria Climent[4], Guiomar Bautista[5], José Alcamí[1,3], Juan Luis Steegmann[6], Montserrat Plana[4], José M. Miró[3], Vicente Planelles[7], María Rosa López Huertas[1], Mayte Coiras[1]

[1] AIDS Immunopathology Unit, National Center of Microbiology, Instituto de Salud Carlos III, Madrid, Spain, [2] Hematology Service, Hospital Universitario Ramón y Cajal, Madrid, Spain, [3] Infectious Diseases Service, Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Barcelona, Spain, [4] Retrovirology and Viral Immunopathology Laboratory, IDIBAPS, Hospital Clínic, University of Barcelona, Barcelona, Spain, [5] Hematology Service, Hospital Universitario Puerta de Hierro, Madrid, Spain, [6] Hematology Service, Hospital Universitario La Princesa, Madrid, Spain, [7] Division of Microbiology and Immunology, Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah, USA

Background: Tyrosine kinase inhibitors (TKIs) are successfully used in clinic for treating chronic myeloid leukemia (CML). Our group described that TKIs show antiviral effect against HIV-1 by interfering with SAMHD1 phosphorylation (pSAMHD1), proviral integration and transcription and decreasing viremia and reservoir size in vivo in humanized NSG mice. TKIs might also impede reservoir maintenance by blocking homeostatic proliferation. Finally, TKIs may induce a deep molecular response (DMR) against cancerous cells, allowing treatment withdrawal in CML patients that nevertheless do not relapse. Objective: to evaluate if CD4+ T cells from CML patients that withdrew TKI treatment were still resistant to HIV-1 infection.

Methods: Expression markers of PBMCs isolated from CML patients that stopped TKI treatment due to DMR (Off-TKI) (n=17) and from healthy donors (n=30) were analyzed by flow cytometry. Proviral integration was analyzed by Alu-qPCR. Viral protein synthesis was quantified by chemiluminiscence.

Results: 1) Off-TKI patients were 57% male, 43% female; CML diagnosis mean age was 61±5.5y; mean lymphocyte count was 2.4±0.3×103/ml; previous TKI treatment was with imatinib, nilotinib and/or dasatinib for 5.3±0.4y; mean time without treatment was 1.1±0.2y. 2) In resting conditions, CD4+pSAMHD1+ and CD4+CD25+CD69+ activated cells were reduced 2.1- and 2.6-fold, respectively, in Off-TKI patients, regarding controls. After activation with PHA/IL-2, the expression of these activation markers was quite similar in Off-TKI patients and controls. 3) Proviral integration and transcription was reduced 12.4- and 5.2-fold, respectively, in PBMCs from Off-TKI patients after ex vivo infection with NL4-3_renilla. 4) Expression of NK activation marker CD56 was increased >5-fold and cytotoxic cells CD56+CD16+CD107a+ and CD8±TCRgd+ were increased >5- and 3-fold, respectively, in Off-TKI patients.

Conclusions: Although the expression of activation markers in CD4+ T cells isolated from Off-TKI patients was downregulated at basal level, no differences with controls were found after in vitro activation. However, these cells were resistant to HIV-1 proviral integration and transcription. A parallel increase of cytotoxic cell populations was observed in these patients, suggesting that transient use of TKIs in HIV infected patients may induce a sustained antiviral response that would interfere with reservoir formation, replenishment and reactivation.

PP 6.8.9

Antagonism of PPARγ for Th17 mucosal immunity restoration and HIV reservoir purging

D. Planas[1], A. Fert[1], Y. Zhang[1], M.J. Ruiz[1], J.P. Goulet[2], T.R. Wiche Salinas[1], E.A. Cohen[3], J.P. Routy[4], N. Chomont[1], P. Ancuta[1]

[1] Centre de recherche du CHUM, Montreal, Canada, [2] Caprion, Montreal, Canada, [3] Institut de Recherches Cliniques de Montréal, Montreal, Canada, [4] McGill University, Montreal, Canada

Background: Th17-polarized CCR6+RORγt+CD4+ T-cells are key players in mucosal homeostasis. Th17 cells are preferential targets for HIV infection at mucosal sites and their depletion is not restored even upon early initiation of antiretroviral therapy (ART). Moreover, Th17 cells carrying replication-competent HIV persist during long-term ART. Therefore, novel Th17-targeted HIV remission/cure strategies are needed. Considering that PPARγ represses RORγt, the Th17-specific master regulator, and HIV transcription, we hypothesized that PPARγ pharmalogical inhibition will enhance Th17 functions and facilitate HIV reactivation from latency.

Methods: Total/CCR6+/CCR6- memory CD4+ T-cells from HIV-controls were stimulated via CD3/CD28, exposed to transmitted founder HIVTHRO, and cultured in the presence/absence of the PPARγ antagonist T0070907 for 12 days. Short/long-term viral outgrowth assays were performed in ART+HIV+ in the presence/absence of T0070907 and/or antiretroviral drugs. Cell-associated (CA)/free HIV RNA/DNA and HIV-p24 levels were quantified by real-time PCR, ELISA, and flow cytometry. Transcriptional profiling was performed using the Illumina RNA Sequencing technology.

Results: While PPARγ antagonism increased IL-17A and CA HIV RNA levels in cells of ART-treated PLWH, viral outgrowth was unexpectedly inhibited. To define the mechanism of action, RNA-sequencing was performed. PPARγ inhibition in CCR6+ T-cells up-regulated transcripts linked to Th17 polarization (RORγt, STAT3, BCL6 IL-17A/F, IL-21), HIV transcription (CDK9, HTATIP2) and restriction (Caveolin-1, TRIM22, TRIM5α, BST2, miR29), and down-regulated transcripts encoding HIV-dependency factors (CCR5, furin). Moreover, T0070907 increased the antiviral IL-21/miR29 axis.

Conclusions: These Results: provide the rationale for considering PPARγ antagonism as a novel strategy towards Th17-mediated mucosal immunity restoration and HIV-reservoir purging.

PP 6.8.10

Targeting STAT SUMOylation to enhance NK cell cytotoxicity

A. Macedo[1], C. Levinger[1], A.C. Hernandez Santini[2], N. Bonan[1], B. Nguyen[1], K. Crandall[1], R. Lynch[1], A. Bosque[1]

[1] George Washington University, Washington, USA, [2] University of Puerto Rico, Ponce, Puerto Rico

Background: The signal transducer and activator of transcription (STAT) protein family are transcription factors that mediate diverse aspects of cellular immunology. The function of STATs is regulated at the posttranslational level by phosphorylation, acetylation and SUMOylation. Over the last years, our laboratory has been studying 3-Hydroxy-1,2,3-benzotriazin-4(3H)-one (HODHBt). HODHBt is a benzotriazine that blocks SUMOylation of STAT5 increasing its nuclear presence and transcriptional activity in human CD4 T cells. HODHBt leads to hyperactivation of STAT5 mediated by IL-2 and reactivates and decreases latent HIV-1 both in vitro and ex vivo. Because STATs and in particular STAT5 are important transcription factors in the cytotoxicity of natural killer (NK) cells, we wanted to understand whether HODHBt could also enhance NK cell cytotoxicity.

Methods: We have studied whether HODHBt enhances IL-15-induced NK activation in HIV-negative donor NK cells isolated from PBMCs. Our analyses include i) transcriptome profile by RNAseq, ii) expression of activation markers and cytotoxic proteins by flow cytometry and, iii) functional assays to address the NK cytotoxicity.

Results: Our Results: indicate that HODHBt increased phosphorylation mediated by IL-15 of different STATs in NK cells. Transcriptome profile identified 203 differentially expressed (DE) genes between IL-15 and IL-15 plus HODHBt. These DE genes belong to pathways related to cytokine signaling and include cytotoxic molecules like GZMB and GZMA. At the protein level, HODHBt enhanced IL-15-induced expression of GZMB, GZMA, activation markers CD69 and CD25 and IFN-g production among others. Finally, functional studies showed that HODHBt increased NK-cytotoxicity.

Conclusions: Targeting STAT SUMOylation with HODHBt has both latency-reversal activity in CD4T cells and immune enhancer activity of NK cells. Thus, STAT SUMOylation is an interesting candidate pathway to target for shock-and-kill strategies. These dual effects may favor the elimination of latent reservoirs.

PP 6.8.11

Broad-spectrum gRNAs abolish HIV-1 LTR-mediated transcription in cells that receive CRISPR/Cas9 therapy

B. Wigdahl, A. Allen, S. Worell, G. Nwaozo, R. Madrid, W. Dampier, M. Nonnemacher

Drexel University College of Medicine, Philadelphia, USA

Background: HIV-1 persistence is a major hurdle to a cure. Genomic editing with the CRISPR/Cas9 system holds promise to permanently excise or inactivate integrated provirus. Broad-spectrum gRNAs were designed by isolating patient PBMCs, deep sequencing the LTRs, and the most effective gRNAs were selected using a bioinformatic algorithmic pipeline. This resulted in the development of a broad-spectrum gRNA designated SMRT1. SMRT1 demonstrated knock-down of HIV-1 LTR-driven transcription in a transient transfection system. This resulted in a residual amount of LTR-driven transcription that was postulated to be due to a lack of delivery to all cells.

Methods: To further elucidate the nature of the residual LTR-driven transcription, a novel dual florescence system was first designed that used the NL4-3 HIV-1 molecular clone that also encoded GFP while the Cas9 expression system also encoded RFP and the anti-HIV-1 SMRT1 gRNA. Second, a VSV-G pseudotyped NL4-3 GFP was utilized. Third, a highly sensitive beta-galactosidase system was used to examine combined gRNA edits. Finally, the latent J-Lat 10.6 cells were used to examine gRNA edits.

Results: Using the two plasmids, it was shown that when the Cas9 system was active, there was at least a 98 percent reduction in GFP expression. Furthermore, when a VSV-G pseudotyped NL4-3 GFP was used and Cas9 (RFP) was delivered to cells, there was again an extensive reduction in GFP expression. Using the beta-galactosidase system, when multiple gRNAs were used at least a 97 percent reduction in LTR-driven gene expression was observed. To model a latently infected T-cell, the J-Lat 10.6 cell line was used. The J-Lat 10.6 cells were transduced with a lentiviral vector that encodes for Cas9 and a gRNA from the same vector. This experiment showed that T cells which received SMRT1, or a gRNA targeting the viral protein Tat, were the most effective at stopping cells from reactivating from latency.

Conclusions: These studies represent a step towards understanding the complex task of using CRISPR/Cas9 for HIV-1-targeted excision/inactivation therapy.

PP 6.8.12

CRISPR/Cas9 editing of HIV-1 transcription factor binding sites on the 5′ long terminal repeats to permanently inactivate latent provirus

B. Wigdahl, C-H. Chung, A.G. Allen, A.J. Atkins, R. Costello, N.T. Sullivan, M.R. Nonnemacher, W.N. Dampier

Drexel University, Philadelphia, USA

Background: The effective control of human immunodeficiency virus type 1 (HIV-1) replication using antiretroviral therapy (ART) is insufficient to cure the chronic HIV-1 infection. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system have successfully demonstrated the excision of integrated HIV-1 provirus from infected cells. However, relying solely on proviral excision may not provide an effective therapeutic profile due to low frequency of excision. Therefore, gRNAs that target the 5′ LTR in order to introduce sequence edits that may permanently inactivate transcription continue to be sought.

Methods: We developed a computational pipeline informed by the Cutting Frequency Determinant (CFD) matrix to identify gRNA sequences that are predicted to cleave most genetic variants among HIV-1 subtype B LTR sequences in Los Alamos National Laboratory database.

Results: Our computational analysis identified two gRNAs that target the NF-kappaB binding sites (gNFKB1, gNFKB2). When scanning all endogenous NK-kappaB sites, the predicted gRNA binding efficiency showed that none of the human NF-kappaB sites had a cleavage score over 0.19 using the CFD matrix. Conversely, HIV-1 NF-kappaB binding from 5,100 LTR sequences had an average cleavage score of 0.84. Furthermore, GUIDE-seq, which detects CRISPR-induced off-target edits in vitro, showed that the gRNA targeting NF-kappaB binding sites had high efficiency on the integrated HIV-1 in TZM-bl cells with no detectable CRISPR-induced off-target edits in the human genome. Lastly, 5′ LTR-driven HIV-1 transcription reduced 45% with treatment of Cas9/gNFkB1 after post-CRISPR PMA/I stimulation, indicating that the 5′-LTR has been deactivated by CRISPR/Cas9.

Conclusions: These Results: demonstrate a working model to deactivate HIV-1 transcription with high safety by targeting critical viral transcriptional regulatory sites.

PP 6.8.13

AsCpf1/crRNA-array excises HIV-1 proviral genome more efficiently than SaCas9/multiplexed-sgRNAs

W. Hu[1], Y. Zhu[1], E. Bouikidis[1], F. Li[1], Y. Lin[1], Y. Cai[2], H. Wang[1], L. Montaner[2], W. Ho[1], S. Turville[3]

[1] Temple University School of Medicine, Philadelphia, USA, [2] Wistar Institute, Philadelphia, USA, [3] University of New South Wales, Sydney, Australia

Background: CRISPR/Cas editing technology holds great potential for treating infectious diseases such as HIV/AIDS. The most promising and well-studied CRISPR/Cas systems include the Streptococcus pyogenes Cas9 (SpCas9), Staphylococcus aureus Cas9 (SaCas9), Campylobacter jejuni Cas9 (CjCas9), Acidaminococcus species BV3L6 Cas12a (AsCas12a, previously **AsCpf1**) and Lachnospiraceae bacterium ND2006 Cas12a (LbCas12a). Multiplex genome editing using Cpf1 only needs a single CRISPR RNA (crRNA) array, but each guide RNA (gRNA) requires its own promoter if using Cas9. This study was to evaluate whether AsCpf1/crRNA-array could be used as a better HIV-1 proviral eradicator than SaCas9/gRNAs.

Methods: We constructed all-in-one lentiviral vectors (LV) including LV-AsCpf1/crRNA-array and LV-SaCas9/multiplexed-gRNAs. The LV-AsCpf1/crRNA-array contained EF1α-driven AsCpf1 and U6-promoted crRNA array targeting two sites within HIV-1 5′- and 3′-LTR, one within Gag, and one within Pol, while the LV-SaCas9/multiplexed-gRNAs included multiplexed gRNAs targeting HIV-1 LTRs, Gag and Pol. The LV-EGFP encoded green fluorescent protein to be used as control to evaluate the packaging and transduction efficiency. After validation by Sanger sequencing, the standard lentivirus pseudotyped with VSVG or CD4-targeted HIV-1 S8-Env was prepared. Their transduction efficiency and HIV-1 proviral excision were determined using the EcoHIV-luciferase reporter HEK293T cell line as well as Ghost(3) X4R5 and HuT-R5 cell lines.

Results: VSVG-pseudotyped LV-AsCpf1/crRNA-array induced cleavage of HIV-1 genome in EcoHIV-luciferase reporter HEK293T cell line. We observed S8-Env-mediated EGFP transduction efficiency at >90% in Ghost X4R5 and 18% in HuT-R5. Direct-PCR genotyping analysis identified most efficient excision in HIV-1 NL4-3 strain and its derived RGH-Env in both cell lines, while weaker excision of Bal, 89.6 and JR-CSF strains. Different sets of PCR primer pairs identified various patterns of HIV-1 proviral excision. AsCpf1/crRNA-array showed robustly higher levels of HIV-1 excision than SaCas9/multiplexed-gRNAs in both cell lines: For example, 5′-LTR/Gag analysis on NL4-3 sample showed 68% cut efficiency (deletion fragment over uncut wildtype PCR product) in AsCpf1 group but 18% in SaCa9 group.

Conclusions: These data supports that AsCpf1/crRNA-array eradicates HIV-1 genome more effectively than SaCas9/multiplexed-gRNAs. The usage of LV-AsCpf1/crRNA-array for HIV-1 proviral eradication in patient T cells (*ex vivo*) and preclinical animal models (*in vivo*) is expected to further validate this strategy.

PP 6.8.14

Block and kill: a new approach to a prevent HIV reactivation, reduce immnue activation, induce apoptosis of infected cells

A. Garzino Demo[1], S. Lingling[2], C. Cairo[2], T.W. Chun[3], M.K. Lafferty[2]

[1] Institute of Human Virology, University of Maryland School of Medicine, Department of Molecular Medicine, University of Padova, Italy, Baltimore, USA, [2] Institute of Human Virology, University of Maryland School of Medicine, Baltimore, USA, [3] National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, USA

Background: Combination antiretroviral therapy (cART) has reduced the impact of human immunodeficiency virus (HIV) infection, but there is no treatment that can eradicate the virus. A strategy to eliminate HIV-1 through the 'shock and kill' approach relies on effective blockade of HIV spread by cART during the 'shock' phase and the elimination of infected cells by cytotoxic lymphocytes during 'kill' phase. However, neither have been achieved. Alternatively, CCR5 deletion or HIV excision from virus target cells has potential for cure but off target effects and removal of all replication competent proviral DNA currently prevent clinic deployment. In the shorter term, new therapeutic approaches are needed to prevent and decrease immune activation that drive HIV replication. This approach would be useful to both decrease immune activation and block HIV replication, which would be especially desirable in the CNS where a restricted replication is thought to occur constitutively.

Methods: We propose a novel strategy, 'block and kill' consisting in the blockade of MALT1 to reduce both cell activation and HIV replication in infected cells. And induction of apoptosis of infected cells. MALT1 is part of the CARMA1-Bcl10-MALT1 complex, which is induced specifically in lymphocytes by antigen receptor activation, and in macrophages by activation. MALT1 has paracaspase and scaffolding activities that lead to NF-κB activation. MALT1 inhibitors disrupt NF-κB binding to the HIV LTR and abrogate Tat-induced viral transcription. MALT1 is a target for the development of drugs to treat lymphomas. Among MALT1 inhibitors are FDA-approved drugs that have been used to treat psychotic disorders, suggesting that they cross the blood-brain barrier.

Results: We show that currently available MALT1 inhibitors block HIV replication, lack toxicity, and decrease expression of surface activation markers. Further, MALT1 inhibitors block reactivation of HIV expression in both cell lines and primary cells from virally suppressed HIV-infected individuals. We also show that MALT1 inhibition induces apoptosis of infected cells, but not of uninfected cells.

Conclusions: The 'block and kill' approach has merit as a therapeutic modality, in conjunction with cART, preventing HIV reactivation and immune activation and inducing selective apoptosis of infected cells, towards a functional cure for HIV

PP 6.8.15

HIV-1 diversity considerations for clinical studies of passively transferred broadly neutralising antibodies

K. Wagh[1], K. Stephenson[2], D. Barouch[2], B. Korber[1]

[1] Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, USA, [2] Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, USA

Background: Passive delivery of broadly neutralizing antibodies (bNAbs) for HIV-1 therapy is promising because bNAbs are longer lived in vivo and can trigger effector functions for clearing infected cells. However, HIV-1 can readily escape antibody pressure, and the target env gene shows high within-host and global diversity. Here, we discuss the critical requirements such diversity imposes on bNAb-based therapy.

Methods: We used published neutralization data for 374 global viruses against 6 clinically advanced bNAbs, and our previously developed Bliss-Hill model to predict neutralization by bNAb combinations. To analyze viral escape from bNAbs in vivo, we used data from 5 clinical studies of bNAb therapy during analytical treatment interruption (ATI). We characterized within-host diversity of bNAb efficacy by calculating the prevalence of sequence features associated with sensitivity/resistance to each bNAb analyzed in datasets from 6 well-sampled chronically infected individuals.

Results: We found that most bNAbs show heterogeneous neutralization profiles across different M-group subtypes that dominate different geographic regions, e.g. V3-glycan and fusion peptide bNAbs are inactive against the CRF01_AE form that dominates Southeast Asia, and the V2-apex bNAbs are less active against the subtype B dominant in the Americas and Europe. Analyzing studies of bNAb therapy during ATI, we quantified the differences in neutralization sensitivity that allow viral rebound in the presence of single bNAbs in vivo. We also showed that most chronically infected individuals harbor an array of bNAb-resistant viruses at low to moderate frequencies. These factors together suggest that therapy with single bNAbs will be ineffective, and combinations of bNAbs that can neutralize most within-host viruses with at least two bNAbs simultaneously will be required to avoid rapid escape. We next predicted the neutralization profiles of leading 2- and 3-bNAb combinations against a large panel of global viruses, and found that both significantly outperform individual bNAbs. However, a 3-bNAb combination will be needed to achieve coverage with at least two bNAbs active against most viruses in each M-group clade.

Conclusions: HIV-1 diversity, both at the within-host and global scales, places stringent requirements on successful bNAb-based therapy, which may be addressed by combining at least three potent and broad bNAbs targeting different epitopes.

PP 6.8.16

Cleaving HIV-1 provirus from ART-suppressed patient-derived resting CD4 T Cells using Cpf1/crRNA-array ribonucleotide protein packaged by CD4-targeting lentivirus-like particle

W. Hu[1], Y. Cai[2], Y. Zhu[1], F. Li[1], A.O. Stella[3], P. Tebas[4], K. Mounzer[5], J. Kostman[5], S. Turville[3], L. Montaner[2]

[1] Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, USA, [2] HIV-1 Immunopathogenesis Laboratory, Wistar Institute, Philadelphia, USA, [3] Kirby Institute, University of New South Wales, Sydney, Australia, [4] Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA, [5] Philadelphia Field Initiating Group for HIV-1 Trials, Philadelphia, USA

Background: CRISPR/Cpf1 demonstrates advantages over CRISPR/Cas9 because of its multiplexed genome editting using a single CRISPR RNA, crRNA) array. Delivery of CRISPR/Cas9 as preformed ribonucleoprotein (RNP) complex increases editing efficiency and reduces off-target effects. We examined the HIV proviral genome cleavage potential using CRISPR/AsCpf1 RNP packaged by CD4-targeting lentivirus-like particles (LVLP) with peripheral resting CD4+ T cells from HIV-infected ART-suppressed individuals.

Methods: We constructed all-in-one expression vector (LV-Gag/dPol-AsCpf1/crRNA-array) containing EF1α-driven Gag/dPol-AsCpf1 fusion protein and U6-promoted crRNA array. The crRNA-array was designed to target multiple regions of HIV-1 proviral genome (two sites within 5′- and 3′-LTR, one site within Gag, and one site within Pol). The LV-Gag/dPol-AsCpf1/crRNA-array vector was co-transfected into HEK293T cells with packaging vector either expressing vesicular stomatitis virus glycoprotein (VSVG) or human resting CD4 T cell-specific HIV-1 envelope protein (S8-Env) to produce LVLP (noted as V-LVLP and S8-LVLP respectively). LVLP was trasduced into primary resting (CD69-CD25-HLA-DR-) CD4+ T cells isolated from PBMC of HIV-infected ART-suppressed donors (n=9) by spinoculation method. Cells were harvested at 5 days later for DNA preparation. PCR genotyping assay was performed to analyze the excisions of HIV-1 proviral genome following with confirmation using Sanger sequencing method.

Results: The delivery and genome editing efficiency of S8-Env or VSVG-pseudotyped regular lentiviruses were validated using HIV-infected Ghost(3)-X4R5 and HuT-R5 cell lines firstly. Both V-LVLP and S8-LVLP successfully delivered AsCpf1/crRNA-array RNP into ART-Suppressed Patient-derived peripheral resting CD4+ T cells, mediated multiple excisions on HIV proviral genome, and generated various delection patterns (e.g.delection of the regions between 5′-LTR and Gag or between Gag and 3′-LTR). The DNA fragments covering the excision regions from LVLP-transduced cells were confimred by Sanger sequencing and compared to those from the untreated cells. The excised fragments varied in size validating repeat cuts by AsCpf1/crRNA-array RNP when compared to expected cuts using CRISPR/Cas9-induced genome excision.

Conclusions: Our CD4-targeting LVLP is able to deliver Cpf1/crRNA-array RNP into primary resting CD4 T-cell and cleave HIV-1 provirus genome. These data provide a proof-of-concept for the potential usage of Cpf1/crRNA-array RNP as an improved gene editing strategy for the eradication of the HIV reservoir *in vivo*.

PP 6.8.17

A bispecific antibody that simultaneously recognises the V2- and V3-glycan epitopes of the HIV-1 envelope glycoprotein

M. Davis-Gardner, M. Gardner, M. Farzan

Scripps Research Institute, Jupiter, USA

Background: Broadly neutralizing antibodies (bNAbs) can prevent and control an HIV-1 infection, but their breadth is invariably too limited for use as monotherapy. To address this problem, bi- and tri-specific antibody-like constructs have been developed. These engineered antibodies typically have greater breadth than the native bNAbs from which they were derived, but they are not more potent because they do not, in most cases, simultaneously engage more than a single epitope of the HIV-1 envelope glycoprotein (Env).

Methods: Here, we describe a new class of bispecific antibodies targeting the V2-glycan (apex) and V3-glycan regions of the HIV-1 envelope glycoprotein (Env). Bispecific molecules targeting multiple HIV-1 epitopes were constructed using knobs-into-into holes mutations to facilitate proper heterodimerization. These constructs were then compared to their parental antibodies in a pseudovirus TZM.bl neutralization assay against a global panel of HIV-1 isolates.

Results: Bispecific antibodies with a single-chain (scFv) form of the CAP256.VRC26.25 V2-glycan (apex) antibody on one antibody arm and a full V3-glycan Fab on the other arm neutralizes more HIV-1 isolates than the bNAbs from which they were derived. Specifically, bispecific antibodies with a single-chain (scFv) form of the CAP256.VRC26.25 V2-glycan (apex) antibody on one antibody arm and a full V3-glycan Fab on the other arm neutralizes more HIV-1 isolates than the bNAbs from which they were derived. Moreover, these bispecific antibodies are markedly more potent than their parental bNAbs because they simultaneously engage both the apex and V3-glycan epitopes of Env.

Conclusions: Our data show that simultaneous engagement of two critical epitopes of a single Env trimer can markedly increase the potency of a bispecific antibody. Such molecules will be useful in a clinical setting because they will limit viral escape during treatment.

PP 6.8.18

Exploring sequence specific silencing of latent HIV using CRISPR interference

R. Schwarzer, M. Montano, W.C. Greene

Gladstone Center for HIV Cure Research, San Francisco, USA

Background: Hitherto, all broadly applicable therapeutic approaches aiming to neutralize HIV reservoirs have missed their mark. Latency reversing agents, utilized in the context of a shock and kill approach often lack efficacy *in vivo* or induce undesirable and sometimes detrimental side effects. New therapeutic strategies are urgently needed, which may complement or replace current cure strategies.

We are particularly interested in exploring sequence-specific transcriptional silencing of HIV proviruses using clustered regularly interspaced short palindromic repeats interference (CRISPRi). CRISPRi does not involve gene excision, but instead exploits a catalytically inactive dCas9 protein that is fused with transcriptional repressors, thus neutralizing the promotor activity of the proviral LTR by introducing repressive marks on chromatin and DNA.

Methods: Silencing of viral gene expression was established and thoroughly tested in different cell line models of HIV latency. CRISPRi delivery was achieved by either lentiviral transduction or nucleofection thus enabling permanent or transient silencing modes respectively, which were monitored throughout cell division and upon T cell activation.

Results: We have designed a panel of single guide RNAs (sgRNAs) that target dCas9 silencing complexes to the viral LTR and efficiently inhibit viral gene expression upon broad T cell activation or viral reactivation with latency reversing agents. Several highly active sgRNAs were identified that almost completely suppress viral transcription in the J-Lat 5A8 and other cell line models of HIV latency. In Jurkat cells, the CRISPRi system efficient halts viral gene expression in the context of a previously established, ongoing viral replication and blocks HIV transcription in newly infected cells. We found that a transient expression of CRISPRi only leads to a temporary suppression of HIV. Therefore, we devised a lentiviral delivery vector that enables a Tat-dependent CRISPRi expression, thus confining the expression of the suppressor complex to HIV infected cells.

Conclusions: CRISPRi represents a promising approach for silencing HIV transcription even in presence of strong activating stimuli. Importantly, our CRISPRi approach obviates double strand breaks that are induced by catalytically active Cas9, thus reducing the risk of oncogenesis and other detrimental side-effects and may be further developed into a sequence-specific block and lock strategy.

PP 6.8.19

Enhancement of antibody-dependent cellular phagocytosis is essential to the clearance of HIV-1 reservoirs in lymphoid organs

L. Shan

Washington University, St. Louis, USA

Background: Current approaches to purging the HIV-1 latent reservoirs in CD4+ T cells involve pharmacological reactivation of HIV-1 transcription and induction of viral-specific host immune responses. Antibodies targeting HIV-1 envelope protein can mediate killing of infected cells through ADCC and ADCP. Lymphoid organs function as major reservoirs for HIV-1. However, most NK cells in lymphoid organs do not express Fc receptors and are functionally impaired during chronic infection. Therefore, ADCP is important to clear residual viruses in lymphoid tissues. SIRPα is an inhibitory receptor on phagocytes that engages with CD47, which is ubiquitously expressed. Blocking SIRPα and CD47 interaction may enhance phagocytosis of HIV-1-infected cells.

Methods: For in vitro experiments, NK cells and MDMs were co-cultured with autologous CD4+ T cells infected with HIV-1 reporter viruses. HIV-1 antibodies were used to trigger ADCC or ADCP. We also used a novel humanized mouse model named MISTRG, in which human cytokine coding genes including M-CSF, GM-CSF, IL-3 and IL15 are knocked into their respective mouse loci. This novel mouse model develops functional human macrophages and NK cells.

Results: In HIV-BaL infected mice treated with neutralizing or non-neutralizing antibodies, frequency of HIV-1-infected cells reduced in non-lymphoid tissues. No reduction was observed in spleen, due to the lack of spleen NK cell functions. To boost ADCP, we tested SIRPα-CD47 blockade strategy in tissue culture system and humanized mouse models. In cell culture, 5-20% infected cells were phagocytosed when treated with HIV-1 antibody alone. Combination with anti-SIRPα antibodies enhanced ADCP function which led to clearance of more than 90% infected cells. In HIV-BaL infected mice, PGT121+anti-SIRPα combination significantly lowered plasma HIV-1 RNA levels compared to PGT121 alone and control groups. Alghout PGT121 alone reduced HIV RNA levels in non-lymphoid tissue, reduction of HIV-1 RNA and frequency of p24+ cells in the spleen was only achieved with PGT121+anti-SIRPα combination.

Conclusions: In summary, our study demonstrates that clearance of HIV-1-infected cells by ADCC is not efficient in lymphoid tissues. CD47-SIRPα blockade can enhance ADCP of HIV-1-infected CD4+ T cells and can efficiently clear viral infection in lymphoid organs. Our study provides critical implications to HIV cure research.

PP 6.8.20

Tat inhibition by didehydro-cortistatin A promotes heterochromatin formation at the HIV-1 long terminal repeat

C. Li, S. Valente, G. Mousseau

Scripps Research Institute, Jupiter, USA

Background: The HIV-1 Tat protein regulates the passage from viral latency to active transcription by binding to the viral mRNA hairpin (TAR), and recruiting transcriptional factors to the HIV promoter to promote transcriptional elongation. The viral promoter is directly governed by its chromatin environment, and the nucleosome-1 downstream from the transcription start site directly impedes transcription from the HIV-1 promoter. The Tat inhibitor Didehydro-Cortistatin A (dCA) inhibits HIV-1 transcription and overtime the lack of occasional low-grade transcriptional events Results: in the accumulation of epigenetic marks at the latent loci that 'lock' HIV transcription in a latent state.

Methods: To examine the molecular correlates that controlled transcription from the HIV-1 promoter in response to long-term dCA treatment, we assayed histone density and chromatin accessibility using four different HIV cell models with different transcriptional strengths: HeLa-CD4 cells chronically infected cells with NL43, were used as our model of high transcriptional activity; the promyelocytic OM10.1 cell line was used as a latent model with low transcriptional activity; U1 cell line containing proviruses with mutations in Tat, was used as our model of suboptimal Tat activity; and finally ACH-2 cells with mutations in TAR, and unresponsive to Tat, as our Tat transcriptional null model.

Results: We demonstrated that dCA treatment does not alter the classic nucleosome positioning at the HIV-1 promoter, but promotes tighter nucleosome/DNA association correlating with increased H3 occupancy at Nuc-1 associated with decreased Histone acetylation. The recruitment of SWI/SNF chromatin-remodeling complex PBAF, required for Tat activation of the HIV-1 promoter, was also decreased; while its silencing counterpart, BAF, was increased at the HIV promoter in dCA treated cells. These Results: were supported by loss of RNA polymerase II recruitment on the HIV genome, even during strong stimulation with latency-reversing agents. No nucleosome/DNA association changes were detected in cell line models of latency with Tat-TAR incompetent proviruses, demonstrating the specificity of dCA activity.

Conclusions: We characterized the dCA-mediated epigenetic signature on the HIV promoter, which translates into potent blocking effects on HIV expression. This study highlights the advantage of the introducing Tat inhibitor in in 'block-and-lock' eradication strategies.

PP 6.8.21

Resistance to the Tat inhibitor didehydro-cortistatin A is mediated by heightened basal HIV-1 transcription

S. Mediouni, S. Valente

Scripps research Institute, Jupiter, USA

Background: HIV-1 Tat enhances viral RNA transcription by binding to the viral RNA structure TAR and recruiting transcriptional cofactors. Tat enhances its own transcription via a positive feedback loop. Didehydro-Cortistatin A (dCA) is a potent Tat inhibitor (EC50=1nM), that reduces HIV-1 transcription promoting a state of persistence latency, refractory to viral reactivation. dCA has shown very promissing in 'block-and-lock' functional cure approaches. Here, we investigated the viral genetic barrier to dCA resistance in vitro.

Methods: Viruses resistant to dCA were obtained by passaging the virus NL4-3 in increasing concentrations of dCA over a 12-month period. We identified 2 isolates resistant to 1μM dCA. Mutations were identified using Next generation Sequencing and a detailed work using chimeric viruses identified key resistance features.

Results: Mutations in Tat and TAR were not identified, consistent with the high level of conservation of these elements. Instead, viruses resistant to dCA developed higher Tat-independent basal transcription. We identified a combination of mutation in the HIV-1 promoter that increased basal transcriptional activity, and modifications in viral Nef and Vpr protein that increased NF-kB activity. Importantly, this heightened transcriptional activity renders CD4+ T cells infected with these viruses more susceptible to cytotoxic T cell-mediated killing and to cell death by cytopathic effects.

Conclusions: This is the first report of viral escape to a Tat inhibitor resulting in heightened Tat-independent activity, all while maintaining wild-type Tat and TAR. Results: provide insights on drug resistance to a novel class of antiretrovirals and reveal novel aspects of viral transcriptional regulation.

PP 6.8.22

HLA-E-presented peptides as novel targets for HIV-1 therapy

S. Brackenridge[1], H. Yang[1], D. Li[2], G. Gillespie[1], B. Haynes[2], A. Mcmichael[1]

[1] University of Oxford, Oxford, UK, [2] Duke University, Durham, USA

Background: A Rhesus CMV SIV vaccine induces a broad, atypical SIV-specific CD8 T cell response that allows ~50% of vaccinated Rhesus macaques to clear infection following challenge with SIVmac239. Two thirds of these CD8 responses are restricted by MHC Class II, the remainder by MHC-E. In contrast to the limited MHC-E peptide repertoire described previously, the Mamu-E-restricted SIV epitopes exhibit surprising sequence diversity. HLA-E exhibits limited polymorphism, and is expressed on nearly all cell types, with high-level expression on T follicular helper cells – an important reservoir of HIV-1. Our goal is to produce reagents specific for MHC-E presenting HIV-1 epitopes and to evaluate their potential as therapeutic agents for the treatment of HIV-1.

Methods: HLA-B27/β2-microglobulin transgenic mice were immunized with disulphide-trap-stabilized HLA-E/peptide/β2-microglobulin complexes, and monoclonal antibodies (mAbs were isolated by spleen cell fusion or single cell PCR of B cells sorted with HLA-E tetramers. Human mAbs were isolated from healthy donors using single B cell tetramer sorting and PCR. MAb variable regions were engineered into human IgG backbones and their specificity assessed by staining cells expressing single chain trimers (SCT) of peptide, β2-microglobulin and HLA-E heavy chain. T cell clones specific for HLA-E presenting HIV-1 peptides were isolated by in vitro priming and tetramer sorting.

Results: A number of antibodies recognising HLA-E presenting the HIV-1 Gag RL9 epitope have been isolated. One of these, CA117, recognises HLA-E presenting both the B and C clade RL9 peptides (RMYSPTSL and RMYSPVSIL), but not the SIV Gag equivalent (RMYNPTNIL), the canonical VMAPRTLLL peptide, or two Mycobacterium tuberculosis epitopes (RLPAKAPLL and RMAATAQVL). This specificity implies it will be suitable for development as a therapeutic reagent. T cell clones, specific for HLA-E presenting the RL9 Gag epitope, have also been isolated and shown to kill HIV-1 infected cells in vitro. We are producing recombinant forms of their T cell receptors (TCRs) to determine whether they will have potential as soluble therapeutic reagents or for development of CAR-T cells.

Conclusions: The HLA-E-specific mAbs and TCRs that we are generating may have utility for exploitation in future therapeutic strategies against HIV-1.

PP 6.8.23

Development of anti-PD-1 chimeric antigen receptor T cells to target a PD-1+ CD4 T cell population enriched in HIV provirus

K. Eichholz[1], F. Haeseleer[2], L. Corey[3]

[1] VIDD, Fred Hutchinson Cancer Research Center, Seattle, USA, [2] Departments of Laboratory Medicine and Medicine, University of Washington, Seattle, USA, [3] Departments of Laboratory Medicine and Medicine, University of Washington, VIDD, Fred Hutchinson Cancer Research Center, Seattle, USA

Background: T cell-based immunotherapies that use a chimeric antigen receptor (CAR) have shown impressive clinical success to treat hematologic cancers and revived interest as an effective therapy for HIV. The decentralized distribution of the viral reservoir cells and the paucity of reactivated cells hampers the direct translation of the CAR approaches to treat HIV. PD-1+ CD4 T cells in viremic and ART-treated individuals are enriched with replication-competent HIV proviruses and these cells are responsible for persistent viral transcription on ART. Here, we engineered and tested aPD-1 CAR T cells to target an endogenous marker of HIV+ cells.

Methods: We designed 6 CAR-41BB-CD3z-EGFRt expression cassettes (aPD-1 CAR) based on the FDA-approved anti-PD-1 antibody Pembrolizumab with 2 single chain variable fragment orientations (VH-VL and VL-VH) and 3 extracellular polypeptide linker of variable length (long (L), medium (M) and short (S)). CAR expression, tonic signaling of the CAR and signaling in response to PD-1+ cells in transduced Jurkat cells were tested by flow cytometry. Primary rhesus macaque T cells were used for CAR T cell production and cultures were monitored for EGFRt and PD-1 expression and fracticide. CAR T cell killing efficacy of SIVMac239-NefIRES GFP-infected CD4 T cells or K562 PD-1-GFP cells was assessed with a live cell imaging assay.

Results: The 6 aPD-1 CAR exhibit low tonic signaling in resting cells and antigen-specific signaling in presence of PD-1+ cells. Flow cytometry with a fluorescently-labelled PD-1 molecule showed scFv orientation and linker-dependent differences in detectable CAR cell surface expression with the VH VL L aPD-1 CAR showing the highest expression. Viability of primary aPD-1 CAR T cells was not affected by the CAR and PD-1 expression was not detectable during cell production. In preliminary killing assays, all aPD-1 CAR T cells killed infected CD4 T cells and K562 PD-1 GFP cells at various E:T ratio.

Conclusions: We engineered aPD-1 CARs with low tonic signaling that kill SIVmac239-infected and other PD-1+ cells. Further investigation into antigen-induced proliferation, cytokine profiles and performance in 'serial killing' assays are required to choose a lead construct before proceeding into animal experiments.

PP 6.8.24

Conditions for post-rebound SHIV control in autologous hematopoietic-stem cell transplantation

F. Cardozo[1], E. Duke[1], C. Peterson[1], D. Reeves[1], B. Mayer[1], H.P. Kiem[1,2], J. Schiffer[1,2]

[1] Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [2] University of Washington, Seattle, WA, USA

Background: Autologous hematopoietic stem and progenitor cell (HSPC) transplantation with CCR5 gene-edited cells is a promising strategy for achieving sustained HIV remission. However, only some HSPCs can be edited ex vivo during autologous transplantation. We sought to determine the conditions required in autologous HSPC transplantation for viral control in SHIV-infected pigtailed macaques after analytical treatment interruption (ATI) using mathematical modeling.

Methods: We analyzed data from 22 SHIV-1157ipd3N4-infected juvenile pig-tailed macaques treated with combination antiretroviral therapy (cART) 6 months after infection: 5/22 control macaques remained on cART with no further intervention until ATI; 17/22 underwent conditioning with total body irradiation (TBI) and autologous HSPC transplantation during cART with (12/17) and without (5/17) ex vivo CCR5 gene editing using a zinc finger nuclease platform. We developed mathematical models to recapitulate the observed kinetics of CCR5+CD4+, CCR5-CD4+, and CD8+ T cell counts and SHIV plasma viral loads in control and transplanted macaques before and after ATI. After model validation and selection, we generated model simulations of T cell and SHIV dynamics after transplantation and ATI using varying percentages of CCR5-edited/protected from SHIV.

Results: The best-fit model predicts that following autologous HSPC transplant: slow thymic export is the main driver of CD4+CCR5– T cell growth, and rapid lymphopenia-induced proliferation of remaining cells (rather than transplanted cells) after conditioning is the main driver for CD4+CCR5+ and CD8+ T cell expansion. The model also predicts that autologous HSPC decreases the immunologic response to SHIV: animals with greater loss had a higher viral set point and more profound depletion of CCR5+CD4+ T cells. Further, model projections of those with a loss of immunologic control predict that higher fractions of gene-edited cells are required than those with no immune change.

Conclusions: Our Results: provide a framework to estimate the percentage of CCR5-edited, autologous HSPC necessary to achieve post-rebound control in vivo, and suggest that the addition of virus-specific immunologic approaches may reduce this threshold.

Index