Abstracts of the 8th HIV Persistence during Therapy Workshop

Session 1: Basic science of HIV latency I

OP 1.0

Understanding persistence of the latent reservoir

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Background: Accurate measurement of the latent reservoir is essential for the evaluation of cure strategies. Widely used PCR strategies mainly detect defective proviruses and vastly overestimate reservoir size while the viral outgrowth assay and other assays that measure viral RNA, protein or virion production following a single round of T cell activation miss proviruses that are only induced after multiple rounds. Therefore we developed a novel approach to reservoir measurement that directly quantitates intact proviruses that are capable of causing viral rebound.

Methods: A large database of full genome sequences was used to design a digital droplet PCR assay that directly and separately quantitates intact and defective proviruses.

Results: This approach gives infected cells frequencies that correlate well with results of full genome sequencing. Application to interesting patient populations has provided new insights into reservoir dynamics.

Conclusions: Direct measurement of all of the intact proviruses capable of causing viral rebound with a novel rapid and scalable assay may provide the best way to evaluate cure strategies targeting the latent reservoir.

OP 1.1

HIV-1 proviruses which are integrated into cancer-related genes are inducible

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Background: A significant proportion of the clonally expanded HIV-1-infected cells contain replication competent and inducible HIV-1 proviruses. Cells containing HIV-1 proviruses can undergo clonal expansion through homeostatic proliferation, antigen-induced proliferation or aberrant proliferation through HIV-1 integration into cancer-related genes. Because of technical challenges to identify HIV-1 integration sites and full-length sequences simultaneously, it remains unclear whether HIV-1 proviruses which are integrated into cancer-related genes in the clonally expanded cells are intact or defective. We hypothesize that HIV-1 which are integrated into cancer-related genes are inducible. Cells which contain inducible proviruses and which have the capacity to undergo HIV-1-driven clonal expansion would be a major barrier to cure.

Methods: We developed a novel method termed the HIV-1 RNA SortSeq to identify HIV-1-infected cells containing inducible HIV-1 for sequence analysis. Resting CD4+ T cells from aviremic HIV-1-infected individuals on suppressive antiretroviral therapy (ART) were treated with PMA/ionomycin for 18 hours in the presence of ART to induce HIV-1 RNA expression without ex vivo cellular proliferation. Cells were fixed, permeabilized, and hybridized with HIV-1-specific RNA fluorescent probes. Cells expressing HIV-1 RNA were identified through flow cytometry and sorted for subsequent RNAseq after DNase treatment. Customized pipelines were developed to identify HIV-1 and host genome RNA sequences.

Results: HIV-1 RNA SortSeq identifies cells from HIV-1-infected individuals containing inducible HIV-1, which express readily detectable HIV-1 RNA. We found that in cells showing positive HIV-1 RNA on flow cytometry, some RNA transcripts contain both cancer-related genes and HIV-1 LTR in the same read, indicating the presence of HIV-1 RNA read-through from HIV-1 integration into cancer-related genes. Strikingly, we found host-HIV-1 chimeric RNA which contains host exon of cancer-related genes splicing into known HIV-1 splice acceptors, showing that that HIV-1 which are integrated into cancer-related genes may produce host-HIV-1 chimeric transcripts encoding a novel open reading frame.

Conclusions: We developed HIV-1 RNA SortSeq which can identify HIV-1 integration sites of inducible HIV-1. We found that some HIV-1 integrated into cancer-related genes are actually inducible. Thus, HIV-1 integration may change the cellular transcriptional landscape, and cells containing HIV-1 integration into cancer-related genes represent an important target for HIV-1 cure strategies.

OP 1.2

The contribution of memory CD4+ T cell subset phenotype to latency reversal efficiency

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Background: The latent HIV reservoir persists in individuals on ART predominantly in memory CD4+ T cells, a heterogeneous population comprised of central memory (CM), transitional memory (TM) and effector memory (EM) subsets. Current HIV eradication strategies that aim to reverse latency in this heterogeneous pool of cells have had limited success.

Methods: To characterize HIV latency reversal in all memory CD4+ T cell subsets that contribute to the HIV reservoir in vivo, we developed LARA (Latency and Reversion Assay), a primary cell based in vitro model of HIV latency. To identify pathways associated with latency reversal in each subset, we exposed latently infected cells from both HIV-infected individuals and LARA to different classes of latency reversing agents (LRAs).

Results: Memory subsets showed distinct responses that resulted in varying efficiencies to the LRAs tested. Importantly, the most effective LRAs triggered the differentiation into cells that expressed an EM phenotype. Transcriptional profiling of CD4+ T cells from HIV-infected individuals exposed to bryostatin, the LRA that showed the highest latency reversal, identified several EM specific pathways that were significantly upregulated in both the CM and EM subsets, including genes encoding for cytokines and effector molecules such as IFN-γ, IL-2, IL-4, and TNF.

Conclusions: Together, these results support LRA exposure triggering differentiation toward an EM subset phenotype to be linked to higher latency reversal efficiency. Identification of these pathways is a critical prerequisite to understand factors that influence latency reversal in vivo as well as contributing to the most effective design of regimens capable of comprehensive reactivation of the HIV reservoir in eradication strategies.

OP 1.3

Identification of a promising new class of latency reversing agents

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Background: Despite long-term administration of antiretroviral therapy (ART), HIV-1 persists in a broadly distributed latent reservoir

mainly comprised of resting CD4 T cells. Cells harboring latent provirus typically display little to no HIV-1 gene expression and thus remain invisible to the immune system. To achieve a sustained viral remission in HIV-infected patients off ART, it will be important to both reduce the size of the reservoir and to control viral rebound by eliciting an effective immune response capable of restraining viral spread from the smaller reservoir. One approach involves the combined use of potent and safe latency-reversing agents (LRAs) and a therapeutic vaccine. Our recent studies have shown that activators of the AKT/mTOR pathway form a promising group of LRAs.

Our interest has focused on two small molecules, SB-216763 and Tideglusib that commonly inhibit glycogen synthase kinase-3 (GSK-3). This inhibition results in sequential activation of mTORC2, AKT, and mTORC1. Both compounds are known to have high tissue penetration, including entry into the brain.

Methods: Following treatment of CD4 T cells with the LRAs, we quantified virus production with digital droplet PCR. We assessed viability and T cell activation with flow cytometry, and we characterized the molecular mechanism of these two inhibitors using a combination of immunoblotting, phosphoproteomics and RNA sequencing.

Results: We found that SB-216763 and Tideglusib potently activate latent HIV-1 in both a tissue-based model of latency, and in CD4 T cells isolated from HIV-infected donors on ART.In some experiments these agents are more potent than anti-CD3/anti-CD28 antibodies and latency reversal is not associated with T cell activation as measured by changes in CD69 and CD25 expression. Finally, in contrast to the undesirable compromise of CTL/NK function associated with many first generation LRAs, GSK-3 inhibitors have been reported to enhance CTL and NK cell effector function.

Conclusions: In summary, our findings reveal GSK-3 inhibitors as a new class of potent, safe, non-cell activating LRAs. Next, it will be key to define their activity in vivo using SIV-infected macaques. The fact that Tideglusib is already in phase IIb human trials for myotonic dystrophy and autism could accelerate its testing in HIV-infected patients.

OP 1.4

Single cell analysis of HIV latency reveals diverse proviral and host cell behaviour

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Background: The latent reservoir is inherently diverse with each infected cell exhibiting a potentially unique combination of integration site, epigenetic modifications, and host cell phenotype.However, most studies of HIV latency have relied on assays of bulk cultures in which information about the behavior of individual cells is lost.As such, the application of single cell level methods to HIV latency model systems may reveal previously unappreciated levels of heterogeneity.We hypothesized that latently infected cells exhibit diverse characteristics with respect to proviral reactivation and host cell phenotype, and that characterizing this diversity will be important for clearing the latent reservoir.

Methods: We have characterized a cell line model and a novel primary cell model of HIV latency with two single cell assays – single cell qPCR (sc-qPCR) for viral RNA (vRNA), and single cell RNAseq (scRNAseq). These systems were examined both at rest, and after stimulation with two latency reversing agents (LRAs)– vorinostat, and prostratin.

Results: sc-qPCR for vRNA revealed that a subset of latently infected cells transcribe detectable viral RNA in the absence of stimulation, and that stimulation with LRAs induces a wide range of vRNA levels in infected cells. For transformed cell lines, an apparent threshold of ~500 copies of vRNA was required before virally encoded antigen was detected by flow cytometry, while primary cells exhibited a more complex relationship between vRNA and viral protein expression.Compared to prostratin, vorinostat induced lower levels of viral antigen expression, even in cells with equivalent expression of vRNA, suggesting a post-transcriptional block to viral gene expression. Single cell RNAseq of >2000 latently infected primary cells using the 10x Genomics platform revealed diverse transcriptomic profiles within the infected cell population.Interestingly, cells which exhibited the greatest levels of HIV silencing were enriched for a specific set of host genes that define naïve and central memory T cells, suggesting a role for T cell subset identity in the establishment of latency.

Conclusions: Altogether, these data reveal heterogeneous behaviors of HIV proviruses and host cells at rest, and after stimulation with LRAs, and illustrate the power of single cell methods to provide insights into HIV latency.

OP 1.5

CD32 does not mark the HIV-1/SIV latent reservoir

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Background: A recent report by Descours et al. suggests that the surface expression of CD32a marks the replication-competent latent HIV reservoir in CD4+ T cells. However, after significant effort to replicate these findings, we found no evidence to suggest that CD32 marks a CD4+ T cell population enriched with replication-competent virus in HIV-1 infected study participants or SIV-infected macaques on ART.

Methods: The percent of CD32+ CD4+ T cells was measured in samples from participants or macaques and was correlated with viral DNA. CD32+ and CD32- CD4+ T cells were sorted with or without exclusion of CD20+ and CD14+ cells. Viral DNA was measured either by ddPCR or qPCR. CD32-sorted macaque cells were cultured with CEMx174 cells and virus production kinetics were measured over time by qRT-PCR.

Results: Briefly, CD32high CD4+ T cells were sorted from human PBL, rhesus PBMC, and rhesus LNMC and were not enriched in viral DNA. Additionally, the frequency of CD32high did not correlate with viral DNA content of sorted total CD4+ T cells in blood or tissues. Rhesus CD32high CD4 cells were not enriched in replication-competent virus as determined by viral RNA production after co-culture. We also examined CD32 expression in a cohort of macaques that began ART the day of or 3 days post-infection. There was no difference between groups in CD32 expression on the day of ART initiation, nor after 24 weeks on ART, despite differences in SIV DNA content. We investigated the phenotypes of CD32high cells and observed a greater proportion of memory subsets and higher activation than CD32neg cells. Importantly, the CD32high population is prone to contamination by non-T cells. We found that sorting CD32high cells without excluding CD14+ and CD20+ contaminants can have a significant impact on measurements of proviral DNA content.

Conclusions: Utilizing samples from HIV-infected participants and SIV-infected macaques on ART, we have assessed if CD32 marks the replication-competent reservoir. While we did detect CD4+ T cells expressing CD32, our findings contradict the notion that these populations are enriched in latent virus. We found no significant difference in total and replication-competent proviral DNA content between cell populations including, or excluding CD32 fractions.

OP 1.6

Single-cell RNA-Seq reveals transcriptional heterogeneity in latent and reactivated HIV-infected cells

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Background: Despite effective treatment, HIV can persist in part in latent reservoirs, which represent a major obstacle towards HIV eradication. Targeting and reactivating latent cells is challenging due to the heterogeneous nature of HIV infected cells. We used a primary model of HIV latency and single-cell RNA sequencing to characterize transcriptional heterogeneity during HIV latency and reactivation, and to understand transcriptional programs leading to successful reactivation of HIV expression.

Methods: Primary human CD4$^+$ T cells were infected with an HIV-based vector encoding the *green fluorescent grotein (gfp)* reporter gene. Successfully infected (GFP+) cells were sorted by FACS, expanded in culture and allowed to return to a resting, latent state. Latently infected cells were either left untreated (control), or exposed to the histone deacetylase inhibitor SAHA, or to T-cell receptor (TCR)-mediated stimulation. Cells were separated using a fluidic technology and subject to single-cell RNA sequencing (scRNA-Seq). TCR-stimulated cells were also monitored for GFP expression upon reactivation.

Results: Principal component analysis of 224 single cell gene expression profiles identified two distinct clusters of cells, for each condition (untreated, SAHA, TCR). Quantitative analysis of GFP expression of TCR-activated cells further discriminated the two cell subsets, with one cell subpopulation being more susceptible to HIV expression reactivation than the other cell subpopulation. A common set of 134 genes was differentially expressed between these two cell clusters, and was enriched for viral processes, translation regulation, and RNA and protein metabolism. This specific 134-gene signature was also recapitulated in primary CD4+ T cells isolated from HIV+ individuals, revealing a similar transcriptional heterogeneity.

Conclusions: Transcriptional analysis of single cells identified two CD4$^+$ T cell populations that might reflect two distinct resting cellular states, and displaying a different potential for cell stimulation and HIV reactivation. Our results identified for the first time a specific cellular signature, associated with success of HIV reactivation. These data should provide a valuable tool to facilitate the identification of successful latency reversing agents and help designing targeted strategies for purging the HIV latency reservoirs.

PP 1.0

Insights into mechanisms of HIV reactivation from latency using RNA-Seq gene expression profiling in CD4+ T cells and their maturation subsets following treatment with latency reversing agents.

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Background: Identification of human genes with a role in the HIV replication cycle has been an ongoing effort to facilitate therapeutics development. The majority of studies have utilized cell lines and/or lentivirus-driven reporter gene expression. Our goal was to determine which host factors contribute to transcriptional regulation of wild type HIV in primary CD4+ T cells.

Methods: We employed an *in vitro* model of HIV latency to identify genes, whose change in expression correlated with degree of induced HIV RNA expression following treatment with latency reversing agents (LRA). Total CD4+ T cells, or their maturation subsets of naïve (T_N) and central memory (T_{CM}), were treated with SAHA (1 uM, 24 hours) or the DMSO control. Differential gene expression analyses were performed using RNA-Seq (N=4 donors) and the *EdgeR* package (Bioconductor R). Those genes modulated by SAHA were used to query the NCBI Human Interaction Database for candidate genes with known roles in regulation of HIV transcription. A larger sample (N≥10) was examined for correlated responses, by measuring expression of a subset of candidate genes, using droplet digital PCR.

Results: The NCBI database query revealed SAHA induced up-regulation of 21 activators and 12 repressors of HIV transcription; and down-regulation of 17 repressors and 16 activators. Up-regulation of candidate repressor, *HMGA1* had strong negative correlation with HIV reactivation by SAHA (N=11, R=-0.87, p<0.01). Analysis of cell subsets showed that *MED26*, a co-factor of Sp1 activation, was upregulated and *AES*, a proposed HIV repressor, was downregulated to greater extents in T_N cells compared to T_{CM} cells. These data corresponded with the pattern of higher up-regulation of HIV RNA in T_N cells in response to LRA.

Conclusions: Our results are consistent with *HMGA1* and *AES* roles, as HIV transcriptional repressors and *MED26*, as an activator. Gene expression profiling following LRA treatment may aid in validating gene functions in primary cells, previously proposed for regulation of HIV transcription. Also, investigation of LRA-induced gene responses linked with HIV reactivation in T cell maturation subsets will likely have implications in designs of therapeutic strategies to purge the latent reservoir from CD4+ T cells of all maturation stages.

PP 1.1

Whole genome sequencing of single HIV provirus and its proviral integration site for the study of HIV latency

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Background: HIV proviral sequence and its integration site determines in large part whether an individual maintains post-treatment control. However, HIV sequences are very rare compared to human sequences in infected samples, rendering the difficulty in HIV proviral whole-genome sequencing. We developed a droplet microfluidics based method to enrich HIV genome from HIV-infected human samples by millions fold.We sorted and sequenced single HIV genome as well as the integration site for the study of HIV latency.

Methods: JLAT cell (full-length HIV proviruses infected cells) DNA were spiked into human DNA in a ratio of 1:3000 to mimic the infection rate in clinic samples. DNA mixture were then encapsulated into millions of picoliter-volume aqueous droplets with each drop containing only ~1/3000 whole cell DNA molecules. Templates were amplified in individual droplets. Single HIV genome (including human sequences adjacent to HIV genome) containing drops were then identified with specific PCR primers and probes and sorted into individual tubes for sequencing.

Results: We show that we can get high coverage of whole HIV genome sequence at single cell level, which can be useful for studying HIV provirus heterogeneity. In addition to proviral sequence, we are also able to find that the HIV-1 genome are integrated into MAT2a gene of human genome in JLAT (5A8) cells. The ability to identify proviral sequence as well as human sequences adjacent to the proviral integration site at single cell level should be powerful for studying HIV latency.

Conclusions: Studies are ongoing to enrich and sequence HIV genomes in primary samples from people following stopping of antiretroviral therapy who did or did not have HIV rebound. We believe

this will provide a powerful tool to determine the impact of proviral loci on maintaining post-treatment control.

PP 1.2

CD4+ T-cell activation does not lead to expression of latent infection

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Background: Antiretroviral therapy (ART) has greatly reduced the mortality and morbidity associated with HIV infection but fails to eradicate virus from infected individuals. The major barrier to a HIV cure is the persistence of latent infection, where replication competent HIV genomes are stably integrated into long-lived memory CD4+ T-cells without the expression of virus particles. Latency is established early during HIV infection and is maintained for the life of the patient. A previous study in our laboratory demonstrated that antibody-mediated depletion of CD8+ T cells in ART-suppressed SIV-infected rhesus macaques (RM) resulted in the replication of latent viral genomes. This finding suggests that CD8+ T cells are important for the maintenance of viral latency cooperatively with ART. However, it is possible that the blip in virus replication seen after CD8+ T cell depletion was driven by the depletion-mediated homeostatic replication of CD4+ T cells.

Methods: Here, we perform antibody-mediated CD4+ T cell depletion on ART-suppressed SIV-infected RM in order to determine whether the homeostatic CD4+ T-cell proliferation can drive expression of virus particles. We performed anti-CD4 antibody-mediated depletion of CD4+ T-cells in four ART treated RM. All animals controlled virus with 8 months of ART initiation, and were administered with a RM CD4 depleting antibody after 2 months of virus suppression (week 39).

Results: CD4+ T-cells were reduced by 80% (median=79.9%, range=65–89%, week 43) in peripheral blood mononuclear cells (PBMC) during the first month after depletion, and by 20–50% in lymph nodes and rectal mucosa. During CD4+ T-cell reconstitution, there was an increase in Ki67, CD25 and HLA-DR expression on all CD4+ T-cell memory subsets. Importantly, we observed no increases in plasma viral loads (VL) during the depletion or reconstitution phases of the study. There was also no change in CD8 T-cell activation at CD4 depletion or during CD4 reconstitution.

Conclusions: This pilot study confirms that homeostatically driven CD4+ T-cell proliferation alone does not result in expression of latent virus, and that other

PP 1.3

Regulation of HIV-1 replication by Poly (ADP-ribose) polymerase-1 in CD4 T cells.

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Background: Poly (ADP-ribose) polymerase-1 (PARP-1) is a cellular enzyme implicated in multiple biological processes including DNA repair and transcriptional regulation. The role of PARP-1 in HIV-1 life cycle is not clear yet despite the multiple studies that have evaluated its potential implication in different steps of the viral life cycle.

Methods: We determined the susceptibility to HIV-1 infection in CD4 T cells (SUP-T1 cells) lacking or not PARP-1 expression or cells treated with PARP-1 inhibitors. The effect of the PARP-1 inhibitor INH2BP on HIV-1 replication was also evaluated in primary CD4 T cells. Different mutant HIV-1 lacking Env or NFKB LTR regulation were also characterized. HIV-1 replication was tracked by p24 ELISA or FACS analysis of intracellular p24 or HIV-encoded eGFP. Expression of HIV-driven reporter genes was evaluated in cells infected with non-replicating reporter viruses. Proviral formation was accessed by real time PCR and southern blot analyses.

Results: We evaluated the role of PARP-1 in HIV-1 replication in human CD4+ T cells. PARP-1 deficiency increased HIV-1 replication in SUP-T1 cells at low viral doses. Similarly, the PARP-1 inhibitor INH2BP, that eject zinc affecting PARP-1 DNA-binding activity, enhanced HIV-1 replication in CD4 + T cell lines and primary cells. However, nicotinamide mimetic PARP inhibitors did not modify the susceptibility of SUP-T1 cells to HIV-1 infection. The enhancing effect of INH2BP was only noticed when the inhibitor was added at the time of infection but not 1 hour prior or after infection. Considering its short half-life (30 minutes), INH2BP should affect an early step of the viral life cycle. The effect of PARP-1 on HIV-1 replication requires HIV-1 Env since PARP-1 antagonism does not modify the infectivity of VSV-G pseudotyped, Env-deleted HIV-1. Analysis of the mechanism of action indicated that PARP-1 inhibition promoted HIV-1 gene expression but not provirus formation.

Conclusions: In summary, our data indicate that PARP-1 deficiency or inhibition enhances HIV-1 replication and viral gene expression by affecting an Env-dependent event early in the viral life cycle. Potentially, these findings are relevant to viral transmission and latency establishment.

PP 1.4

Integration site-independent enhancement of latency reversal by HIV-1 Nef

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Background: Nef is a crucial accessory protein that enhances HIV pathogenesis, in part through its ability to evade host immunity, but Nef's contribution viral latency reversal remain elusive.Nef is reported to modulate T cell signaling events, which may alter cellular reactivation in the context of latent infection. To investigate this, we examined the reactivation efficiency of latent HIV+ T cell lines harboring functional or defective Nef.

Methods: Latent CEM-A*02 (CLat) T cell clones were generated using NL4.3ΔEnv viruses encoding Nef_{SF2}GFP, Nef_{G2A}GFP, or $Nef_{NL4.3}$-IRES-GFP. The nef gene was subsequently disrupted in selected CLat clones using CRISPR/Cas9. Viral reactivation induced by TNFα, panobinostat and/or prostratin was assessed using flow cytometry to measure GFP or intracellular Gag-p24 expression.

Results: We generated a panel of CLat-Nef_{SF2}GFP [N=11] and CLat-Nef_{G2A}GFP [N=38] clones and tested latency reversal using a combination of LRAs (TNFα, panobinostat and prostratin). In general, viral reactivation was lower in clones encoding defective Nef_{G2A}GFP (GFP_{MFI}=149 [IQR 123-187]) compared to those encoding WT Nef_{SF2}GFP (GFP_{MFI}=375 [272-474]; p<0.0001), resulting in a reduced number of Gag-$p24^+$ cells. Similar results were obtained when these clones were treated with individual LRAs. Consistent with previous literature, we showed that HIV reactivation efficiency was dependent in part on the proviral DNA integration site. To overcome potential bias associated with these differences between clones, we used two pairs of sgRNA (KO_1 and KO_2) to disrupt the Nef gene in four different CLat clones encoding either Nef_{SF2}GFP or $Nef_{NL4.3}$-IRES-GFP. Total reactivation and Gag-p24 expression were reduced in all four bulk-Nef_{KO} cell lines. To investigate further, we generated and characterized Nef_{KO} clones that lacked the ability to downregulate CD4 and HLA-A*02. These KO clones displayed variable reactivation profiles, but notably, the reactivation intensity (GFP_{MFI}) and % Gag-$p24^+$ cells were lower in Nef knockout clones compared to their corresponding parental cell line (see table).

Conclusions: These results highlight a potential role for Nef in modulating viral reactivation from latency in response to LRAs.Additional studies to assess the impact of natural nef sequence variation on this activity are necessary to determine the clinical relevance of this observation.

PP 1.5

A modified viral outgrowth assay incorporating ultra-sensitive P24 measurements

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Background: The quantitative viral outgrowth assay (QVOA) can provide a minimal but reproducible measure of the frequency of true latent HIV infection, reported as infectious units per million cells (IUPM). Such rare events however, require many cells to assay, and current assays are costly and slow. Furthermore, the QVOA under-represents the frequency of replication-competent proviral infection, as no single ex vivo stimulation results in the outgrowth of all proviruses. However, the QVOA yields a reproducible assessment over time of the frequency of replication-competent HIV, a key measure of the efficacy of cure strategies. Thus any efforts to streamline this assay is of high priority.

Methods: We have developed a modified QVOA, the Digital ELISA Viral Outgrowth or DEVO assay which takes advantage of the recently developed SIMOA platform (Quanterix Inc), capable of femtogram detection of HIV p24 protein in contrast to the picogram limitations of traditional ELISA. 8–10×106 purified resting CD4+ T cells from aviremic, ART-treated HIV+ participants were PHA stimulated in limiting dilution in a 96 well-format. CD8-depleted PHA blasts from an uninfected donor were added to half of the cultures and virus allowed to amplify for 8–12 days. P 24 from culture supernatant was measured by SIMOA.

Results: In a total of 6 such assays performed, HIV p24 was detected in the supernatant of cultures as early as day 8 post stimulation. Importantly, DEVO IUPM values at day 8 were comparable or higher than traditional QVOA IUPM values obtained at day 15. Interestingly, DEVO IUPM values were similar with or without the addition of allogeneic CD8-depleted target PHA blasts traditionally used to expand virus.

Conclusions: The DEVO assay uses fewer resting CD4+ T cells, and may provide an assessment in a shorter time than standard QVOA. This assay offers a new platform to quantify replication competent HIV for a variety of applications, such as measuring the frequency of infection in situations where the number of cells available may be limited, evaluating latency reversal agent (LRA) activity, and measuring clearance of infected cells following the addition of autologous immune effectors.

PP 1.6

Regulation of HIV-1 provirus and CD4+ T cell biology by transcriptional coregulators

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Background: Transcriptional regulation of integrated HIV-1 provirus is obligate on host cell molecular biology. Localized chromatin governing proviral expression and CD4 T-cell biology are incompletely understood. Coregulators are readers, writers, and erasers of histone marks, and transcription relies on coregulator fidelity to upstream signals. Indeed, coregulators serve as signal integrators to elicit gene expression programs in response to various cellular stimuli including steroids, hormones, cytokines, nutrients, ions, or metabolites. Thus, coregulators are critical host-cell factors during latency of HIV-1 provirus and T-cell transcription.

Methods: Model cell lines, donor samples, ex vivo T-cell co-culture models, and a coactivator knockout mouse model were used to study the role of coregulators in HIV-1 transcription, latency, and CD4+ T-cell biology.

Results: We found Steroid Receptor Coactivator 3 (SRC-3) regulates retroviral transcription and can be targeted with MCB-613, a potential latency-reversing agent. MCB-613 is synergistic with SAHA and stimulates HIV transcription in latently infected donor cells. We also recently reported that histone arginine methylation by CARM1 is implicated in latency and reactivation of proviral transcription. We now find that the SRC-3 transcriptional coactivator is highly expressed in regulatory T-cells (Tregs) of mice and humans. Public data and donor sampling confirms that SRC-3 is enriched in CD4+ and Treg cells. SRC-3 knockout mice display lymphoproliferation with significantly elevated T-cell populations, consistent with loss of Treg function. Importantly, targeting SRC-3 with novel small molecule inhibitors can relieve Treg suppression of proliferating conventional CD4 T-cells (Tcon) ex vivo and reduce Treg recruitment to tissues in vivo.

Conclusions: Collectively, the results suggest that coregulator activity during immune evasion is an important and targetable component of HIV-1 and cellular latency.

PP 1.7

Developing an *in vitro* model for HIV-1 latency in Tfh cells using tonsillar tissue

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Background: Follicular helper T cells (Tfh) within the germinal centers (GCs) of secondary lymph nodes are major reservoirs for HIV-1 persistence during antiretroviral therapy. Tfh cells normally mobilize to GCs in response to priming by activated antigen-presenting cells and support B cell maturation into plasma cells for antibody production during an immune response. Tfh cell differentiation is transcriptionally regulated by BCL-6 expression, resulting in $CXCR5^+PD\text{-}1^+ICOS^+CD4^+$ T cells that secrete IL-21. Tfh cells are highly permissive to HIV-1 infection, which is facilitated by transfer of HIV from follicular dendritic cells in vivo. GCs are potential sanctuary sites for HIV-infected Tfh cells, because cytotoxic T lymphocytes fail to express critical GC homing receptors.

Methods: To develop a model of HIV latency in primary Tfh cells, we transduced T cell-receptor (TCR)-stimulated $CD4^+PD\text{-}1^+CXCR5^+$ T cells derived from HIV^- tonsil donors with a replication-defective HIV-GFP construct. We then enriched for HIV^+ cells and cultured them under conditions that promote cell quiescence.

Results: Once the cells achieved a nondividing state, expression of HIV-1 Nef and downregulation of both CD4 and HLA-ABC became highly inducible upon TCR stimulation or treatment with latency reversing agents such as vorinostat (a histone deacetylase inhibitor) combined with IL-15. In contrast to our model for HIV latency in primary Th17 cells, treatment with histone methyl transferase inhibitors did not reverse HIV latency, suggesting that epigenetic silencing plays only a limited role in the maintenance of latency in Tfh cells. However, reactivation in HIV-infected tonsillar T cells is still highly dependent upon HIV Tat function, because inducible expression of HIV-1 Nef was restricted to cells expressing activated positive transcription elongation factor b (PTEF-b). Furthermore, treatment with TNF-α, which mobilizes activated NF-κB to the nucleus, also induced HIV reactivation in our tonsillar model.

Conclusions: Our results demonstrate that HIV-1 can establish latency in $CD4^+$ T cells from secondary lymph nodes by a mechanism regulated by HIV-1 Tat, NF-κB, P-TEFb, and histone deacetylation, but may not directly involve histone methylation. This novel Tfh cell model will be invaluable for the study of mechanisms that control HIV latency and the design of strategies to target this important tissue reservoir.

PP 1.8

Histone lysine methyltransferases selectively restrict HIV in central memory T cells

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Background: Histone lysine methyltransferases (HKMTs) are key mediators of epigenetic silencing. Removal of these epigenetic blocks using inhibitors to EZH2, a key component to the polycomb repressive complex 2 (PRC2) or G9a, a methyltransferase induces HIV transcription from latent proviruses. We hypothesized that long-lived central memory cells will have the strongest epigenetic restrictions and therefore methyltransferase inhibitors will have a greater effect on central memory cells compared to other subsets.

Methods: Memory CD4 T cells from three HIV positive HAART treated donors where sorted into central memory (Tcm), effector memory (Tem) and transitional memory (Ttm) T cells using FACS. Each subset were pretreated with either GSK-343 (EZH2 inhibitor) or UNC-0638 (G9a inhibitor) to prevent H3K27 or H3K9 methylation. After pretreatment, IL-15 was added to induce P-TEFb expression and HIV transcription. HIV transcripts were detected using EDITS (Envelope Detection by Induced Transcription-based Sequencing) by nested amplification of HIV Env splice junctions followed by deep sequencing using the Ion Torrent platform.

Results: Inhibition of EZH2 or G9a resulted in an increase of HIV transcription in memory CD4 T cells. When used in conjunction with an HDAC inhibitor (SAHA) or IL-15, proviral induction is synergistically potentiated. After memory T cells were sorted into Tcm, Tem and Ttm and treated with the EZH2 or G9a inhibitor, there was a large induction of HIV transcription in the Tcm population. This became even more pronounced when used in conjunction with IL-15, and reached levels that were compared, or in some cases slightly higher, than induction by TCR stimulation using anti-CD3/CD28 beads.

Conclusions: Inhibition of EZH2 or G9a results in a significant increase of overall HIV transcription in memory T cells. The Tcm population, which are the most long-lived of the memory T cell population, had strong epigenetic restrictions and responded much more strongly to histone methyltransferase inhibitors than the other memory T-cell subsets. The use of histone methyltransferase inhibitors in conjunction with other LRAs could provide a new strategy for the reactivation of the deeply latent proviruses that accumulate in the central memory population, as part of a "shock and kill" strategy.

PP 1.9

Identification of a new factor involved in DNA methylation-mediated repression of latent HIV-1

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Background: DNA methylation is an epigenetic mechanism of HIV-1 latency. The methylation profile of the latent viral 5'LTR is heterogeneous in latency model cell lines and in patient cells in which it increases progressively during cART. Previously, we reported that the DNA methylation inhibitor decitabine (5-aza-2'deoxycytidine) induces different levels of HIV-1 reactivation in latently-infected T cell lines and *ex vivo* patient cell cultures. However, the mechanism of DNA methylation-mediated HIV-1 silencing remains unclear.

Methods: Sodium bisulfite sequencing, EMSAs, ChIP-qPCR assays, RNA interference, GFP fluorescence FACS, p24 ELISA experiments and purification of primary cells from HIV+ patient blood.

Results: To explore this mechanism, we took advantage of two latently-infected J-Lat cell lines (the 8.4 and 15.4 clones) representing distinct integration sites and showed that these two cell lines exhibited similar levels of 5'LTR CpG methylation in basal conditions but different DNA demethylation extents in response to decitabine. Demethylation at CpG dinucleotides following decitabine-induced reactivation of HIV-1 production occurred at specific and reproducible CpG positions that differed depending on the two J-Lat cell lines studied. Interestingly, a site comprising one of this hotspot for decitabine-induced demethylation was shown to bind UHRF1 (Ubiquitin-like PHD and ring finger domain-containing protein 1), only in one of the J-Lat cell line. Treatment with decitabine caused a decreased *in vivo* UHRF-1 recruitment to the 5'LTR. UHRF1 knockdown using RNA interference and pharmacological approaches showed increased levels of HIV-1 production in latently-infected cells and of HIV-1 transcription in *ex vivo* cell cultures from cART-treated aviremic HIV+ patients, respectively.

Conclusions: We have identified UHRF1 as a factor recruited to the HIV-1 5'LTR in a methylation-dependent manner during latency and which plays a functional role in DNA methylation-mediated repression of HIV-1 gene expression. UHRF1 has not previously been identified as a regulator of HIV latency and might thus constitute a new therapeutic target for HIV cure strategies.

PP 1.10

Upregulation of the Nrf2 antioxidant pathway characterizes the transition from productive to latent infection in CD4+ T cells

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Background: HIV-1 infection is associated with oxidative stress exerting epigenetic effects on viral gene expression [Benhar et al. 2016]. However, the molecular mechanisms behind the cellular response to this stress and their relevance for latency are incompletely understood. We analyzed, in primary CD4+ T-cells, the temporal progression of the redox response during transition between productive and latent infection, its possible downstream targets and therapeutic manipulation.

Methods: Productive to latent infection transition was studied in activated CD4+ T-cells infected with wild-type HIV-1 or with a dual color reporter virus [Calvanese et al. 2013]. Cells infected with wild-type HIV-1 were kept in culture for 14 days to revert to a resting state. Transcriptomic profiles were analyzed by microarray and by RNAseq. Expression of antioxidant enzymes/species was further assessed by specific techniques. Single cell (co)localization of viral nucleic acids and host proteins was measured by 3Dimmuno-DNA- or RNA-FISH.

Results: Initially (day 3 post-infection), there were signs of early oxidative stress, such as increased expression of activator 1 of the superoxide generator NADPH oxidase and markers of iron overloading. Intensification of viral replication (days 5–9 post-infection) was accompanied by glutathione depletion and a decrease in the number of the redox-sensitive PML nuclear bodies ($P < 0.001$). This was paralleled by broad activation of the Nrf2-related antioxidant pathways ($P = 0.020$), including thioredoxin/thioredoxin reductase, the NADPH generator G6PD and the quinone detoxifying enzyme NQO1. Upregulation of antioxidant pathways was partially reversed upon transition to latency (day 14) and was accompanied by reformation of PML nuclear bodies. Pharmacological generation of oxidative stress and PML degradation/silencing, respectively, induced partially selective killing of productively infected cells and favored productive over latent infection.

Conclusions: HIV persistence is favored by an antioxidant response to virus-induced redox changes (determining infected cell survival) and a subsequent redox-sensitive nuclear body reorganization (favoring HIV latency).

PP 1.11

Identification of macrophage reservoirs through tropism of HIV-1 envelope

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Background: Despite advances in antiretroviral treatment (ART), eradication of HIV-1 is still not possible due to viral persistence in cell reservoirs. Macrophages express significantly low levels of the CD4 receptor, yet they are still infected. HIV-1 replicates in tissues that are protected from the effects of cART, including some resident tissue macrophages and microglia cells in the CNS, facilitating the presence of persistent viral reservoirs. Since persistent reservoirs are not eliminated during ART, we hypothesize that macrophages may be a source for HIV-1 reservoir in rebound viremia in individuals undergoing analytical treatment interruption (ATI).

Methods: 71, 97 and 122 HIV-1 full-length envelopes were isolated by single genome amplification from three individuals at rebound plasma viremia followed ATI. To generate infectious recombinant viruses, env sequences were cloned into an infectious HIV-1 backbone, followed by transfection of HEK 293T. Monocyte-derived macrophages were infected with Env-recombinant viruses, and fusogenicity was assessed by a FRET-mediated assay. Replication capacity was monitored for 14 days by reverse transcriptase activity. Phylogenetic analysis was performed to evaluate evolutionary relationships existing among these envelopes.

Results: We found that a small population of Env-recombinant viruses was able to fuse efficiently with macrophages. Of the viruses that fused with macrophages, we identified Env-recombinant viruses that were replication competent, some of which were comparable to the level of the macrophage tropic strains ADA and YU2. Phylogenetic analysis showed the presence of several distinct HIV-1 subpopulations. The relatively low diversity within each clade suggests recent diversification from the common ancestor of each clade. This suggests that several HIV-1 subpopulations persisted in the patient in distinct viral reservoirs that were re-activated during rebound.

Conclusions: The main determinant for macrophage tropism is the HIV-1 envelope. Our findings demonstrate that recombinant viruses containing envelopes isolated at rebound after ATI are able to fuse and spread infection to macrophages. Phylogenetic relationships indicate that from the beginning of rebound to sampling there was not enough time for macrophage tropic variants to evolve from T-tropic ones, suggesting that M-tropic variants may constitute part of an independent HIV-1 reservoir.

Session 2: Basic science of HIV latency II

OP 2.1

The HIV-1 antisense transcript AST recruits the polycomb repressor complex 2 to the HIV-1 5′LTR and acts as a viral latency factor

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Background: During latency HIV-1 expression is restricted via epigenetic events including histone methylation that position nucleosomes Nuc-0 and Nuc-1 at the 5′LTR. Enhancer of zeste homolog 2 (EZH2) is a member of the polycomb repressor complex 2 (PRC2) and the dominant histone methyltransferase that suppresses HIV-1 5′LTR activity via trimethylation of lysine 27 on histone H3 (H3K27me3). In higher eukaryotic cells, PRC2 is recruited to the chromain by long noncoding RNAs molecules (lncRNA). How are Nuc-0 and Nuc-1 precisely and invariably positioned at the 5′LTR irrespective of the integration site and orientation? What is the lncRNA that recruits PCR2 to the 5′LTR? An hypothesis is that HIV-1 encodes for its own lncRNA that recruits PRC2 to the 5′LTR, thus establishing latency regardless of the surrounding chromatin. HIV-1 expresses an antisense transcript (Ast) from a promoter located within the 3′LTR.

Methods: We generated cell lines stably transduced with a lentivirus expressing Ast, and we studied its effect on viral replication, on the establishment and maintenance of latency, and on epigenetic regulation of HIV-1 transcription.

Results: We found that Ast is expressed in many infected cell types, including chronically infected cell lines, primary CD4+ T cells infected in vitro, as well as resting memory CD4+ T cells from HIV-1 infected individuals under cART. HIV-1 infection of Jurkat cells stably transduced with a lentivirus expressing Ast results in reduced viral replication. Reactivation of HIV-1 in latently infected Jurkat E4 cells stably transduced with the same lentiviral construct (JE4AST) is significantly reduced, whereas return to latency is accelerated. ChIP assays showed that RNA Pol II is nearly absent at the HIV-1 promoter in JE4AST cells, and that its presence does not increase following stimulation with LRA. On the other hand, EZH2 and H3K27me3 persist at high levels at Nuc-1 in JE4AST cells even after exposure to LRA. Finally, we demonstrate that Ast associates with PRC2.

Conclusions: Altogether, these results show that Ast is an HIV-encoded inducer of viral latency that recruits PRC2 to the 5′LTR, leading to nucleosome assembly and chromatin silencing. Ast could be used to design new therapeutic agents for durable HIV-1 silencing and latency stabilization.

OP 2.2

Majority of the latent reservoir resides in CD32a negative CD4+ T cells

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Background: The persistence of HIV-1 in a stable reservoir in resting CD4+ T cells is the major barrier to curing HIV-1 infection. Cell surface biomarkers that could distinguish cells comprising this latent reservoir from uninfected cells have been lacking. If identified, these biomarkers could significantly enhance progress towards an HIV cure.A recent study (Descours *et al.*, 2017) identified the cell-surface protein CD32a, a low-affinity Fcg receptor not normally expressed on T cells, as a potential marker for the latent reservoir.

Methods: To explore this biomarker, CD4+ T cells were isolated from 6 HIV-1 infected subjects virally suppressed on cART for at least 6 months and sorted for the expression of CD32. CD4+CD32- and CD4+CD32+ T cells were plated and tested for the presence of infectious HIV-1 using the quantitative viral outgrowth assay (QVOA). Additional studies compared viral outgrowth and HIV-1 proviral DNA levels in CD4+ T cells isolated using differing selection methods in order to investigate the possibility that CD32+ CD4 T cells were being removed using the negative depletion method for purifying CD4+ T cells.

Results: In cultures from 6 aviremic subjects where CD32 sorting was performed, no viral outgrowth was detected in CD4+ T cells expressing CD32 using the standard ELISA for HIV-1 p24 antigen. In contrast, CD4+CD32- cultures showed viral outgrowth that was comparable in frequencies previously measured from the same subjects. Using the ultrasensitive p24 assay used in the original report, low levels of p24 were seen in CD32+ cultures, but levels did not increase exponentially over time. Studies using different modes of total CD4+ T cell isolation, including positive selection or negative depletion, showed no difference in viral outgrowth.

Conclusions: We conclude that an enrichment of HIV-1 infected cells is not observed in viral outgrowth cultures of CD4+ CD32+ T cells while CD4+CD32- T cells from the same subjects had expected levels of infected cells. Detection of p24 antigen using ultrasensitive methods may represent defective virus or assay artifacts. Our results demonstrate that CD32 does not specifically mark the latent reservoir, and that additional efforts are needed to identify biomarkers for latently infected cells.

OP 2.3

Brain macrophages in SIV-infected ART-suppressed macaques represent a functional latent reservoir

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Background: A human immunodeficiency virus (HIV) infection cure requires an understanding of the cellular and anatomical sites harboring virus that contribute to viral rebound upon treatment interruption. Despite antiretroviral therapy (ART), HIV associated neurocognitive disorders (HAND) are reported in HIV-infected individuals on ART. Biomarkers for macrophage activation and neuronal damage in cerebrospinal fluid (CSF) of HIV-infected individuals demonstrate continued effects of HIV in brain suggest that the central nervous system (CNS) may serve as a viral reservoir

Methods: Using a simian immunodeficiency virus (SIV)/macaque model for HIV encephalitisand AIDS, we evaluated whether infected cells persist in brain despite ART. Seven SIV-infected pig-tailed macaques were virally suppressed with ART, and plasma and CSF viremia levels were analyzed longitudinally. To assess whether virus persisted in brain macrophages in these macaques, we used a macrophage quantitative viral outgrowth assay (MΦ-QVOA), PCR, to measure the frequency of infected cells and the levels of viral RNA and DNA in brain. Viral RNA in brain of suppressed macaques was undetectable, although viral DNA was detected in all animals.

Results: The MΦ-QVOA demonstrated that the majority of suppressed animals contained latently infected brain macrophages. We also showed that virus produced in the MΦ-QVOAs was replication competent, suggesting that latently infected brain macrophages are capable of reestablishing productive infection upon treatment interruption.

Conclusions: This report provides the first confirmation of the presence of replication-competent SIV in brain macrophages of ART-suppressed macaques and suggests that the highly debated issue of viral latency in macrophages, at least in brain, has been addressed in SIV-infected macaques treated with ART.

OP 2.4

CD4+ T cells expressing CD32 from HIV-1-positive patients are not enriched for proviral DNA

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Background: A recent publication described enrichment of the latent proviral reservoir in resting CD4+ T cells expressing the receptor CD32. Identification of a marker that distinguishes the minority of cells harboring latent HIV-1 provirus from the pool of uninfected CD4+ T cells would represent a crucial scientific advance. Using cells obtained from aviremic HIV-1-positive participants, we hypothesized that CD32 expression would identify a CD4+ T cell population enriched for proviral DNA.

Methods: Resting CD4+ T (rCD4) cells were purified via negative magnetic bead isolation from peripheral blood mononuclear cells obtained from aviremic HIV-1-positive participants (n=6). CD32-positive rCD4 cells were purified via magnetic bead isolation, and quantitative proviral DNA PCR was carried out on CD32-negative, CD32-positive, and total rCD4 cell populations. Changes in CD32 expression upon T cell activation, de novo or reactivated HIV-1 infection were evaluated in rCD4 cells from both HIV-1-positive and negative donors. In parallel, flow cytometric evaluation of T cell activation markers was carried out to assess for co-expression of CD25, CD69 or HLA-DR with CD32. Viral reactivation was quantified in sorted populations.

Results: rCD4 cells from aviremic HIV-1-positive donors expressing CD32 did not contain significantly more proviral DNA than the CD32-negative population or total rCD4 cells (repeated measures one-way ANOVA, P=0.48). CD32 expression did not change with T cell activation, viral reactivation from latency or de novo HIV-1 infection. CD32 expression did correlate with expression of the activation marker HLA-DR (R2=0.99, P<0.001), but not with CD69 or CD25. Viral reactivation did not differ between CD32+, CD32- and total CD4 sorted cell populations (P=0.49).

Conclusions: CD32 expression on CD4+ T cells from aviremic HIV-1-positive participants was associated with the activation marker HLA-DR but did not identify a population of cells enriched for replication-competent proviral DNA.

OP 2.5

The impact of ART duration on the infection of T cells within anatomic sites

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Background: Understanding the impact of antiretroviral therapy (ART) duration on the dynamics of HIV reservoirs is critical for effective curative strategies. Here, we studied changes in the HIV reservoir size over 3–18 years of ART and compared HIV sequences found in T cell subsets to plasma viremia.

Methods: We performed cross-sectional analysis of 1134 HIV p6-RT RNA sequences from pre- and early-ART plasma; and 3963 HIV-DNA sequences from naïve, central (CM), transitional (TM), and effector (EM) CD4+ T cells sorted from peripheral blood (PB), lymph node (LN) and gut tissues from 26 participants on ART for 3–18 yrs: 12 who initiated ART during acute/early (AHI) and 14 during chronic HIV infection (CHI). HIV infection frequencies in anatomic and cellular sites were computed by maximum likelihood statistics. Expansions of identical sequences (EIS) were determined as ≥2 identical HIV-DNA sequences across all cell types from all anatomic sites in CHI group.

Results: In PB, the fold-change in infection frequency per year on ART was similar between AHI and CHI groups across all cell types. For the CHI group, the infection frequency was stable in PB-derived EM cells (fold-change=1.0/yr on ART). However, the odds of a viral sequence belonging to EIS increased in PB, most substantially in EM cells (p=0.007). No substantial change of HIV infection frequency was observed in cells from the gut. In LN, the AHI group had a larger decline in infection frequencies than the CHI group in each cell type (fold-change=0.092–0.48, p-values=0.0056–0.036). Importantly, for the CHI group, LN-derived EM cells contained HIV-DNA sequences that were more often genetically identical to pre- and on-ART plasma HIV-RNA sequences than other LN cell types.

Conclusions: The infection frequency of PB-derived EM cells was stable during 3–18 years of ART but the EIS increased indicating stochastic cellular proliferation and contraction contribute to HIV persistence in these cells. The HIV reservoir declined in the LN, and substantially so in those who started ART during acute infection indicating early ART initiation promotes T cell reconstitution in the LN. However, our data suggest that in LN tissue the replication-competent HIV is more enriched in EM cells.

OP 2.6

CD32+ CD4+ T Cells are HIV transcriptionally active rather than a resting reservoir

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Background: CD32a was suggested as a marker of the replication-competent HIV reservoir. We aimed to comprehensively assess whether CD32 associates with latent or active HIV reservoir.

Methods: CD4+ T cell-surface expression of CD32 and activation markers (CD69, HLA-DR, and CD25) were measured using flow cytometry on 15 fresh and 12 cryopreserved PBMCs from HIV+ individuals on suppressive antiretroviral therapy (ART). Cell-associated HIV RNA and total HIV DNA were measured in isolated CD4+ T cells and in unfractionated PBMCs using qPCR and ddPCR. HIV DNA and HIV RNA were also measured in either FACS sorted CD32+ and CD32- from total or resting CD4+ T cells (n= 9) or CD32 pull downs (n= 2) with Ab conjugated magnetic beads. PMA-induced p24 secretion was measured using ultrasensitive HIV gag p24 ELISA assay. Non-parametric Wilcoxon signed rank and spearman's rank tests were used for statistical analysis.

Results: Levels of CD32 on fresh CD4+ T cells (median 3.95%, IQR 1.32) were significantly higher than on frozen cells (median 0.41%, IQR 0.3). Percentages of CD69+, HLADR+, and CD25+ were significantly higher on CD32+ cells compared to CD32- cells (p<0.0001). Levels of CD32+CD4+ T cells correlated with levels of HLADR+CD4+ T cells and CD25+CD4+ T cells (p<0.05). Cell-associated HIV RNA correlated positively with frequency of CD69+CD32+CD4+ T cells in PBMC (rho=0.65, p=0.01), or isolated CD4+ T cells (rho=0.61, p=0.017). CD4+ T cell-associated HIV DNA correlated positively with the frequency of CD69+CD32+CD4+ T cells (rho=0.53, p=0.044). Enrichment of HIV RNA (total, unspliced, poly-adenylated, and multispliced) was observed in sorted CD32+CD4+ T cells (3.9 to 7.4 fold) with only a slight enrichment (~1.5 fold) in HIV DNA when compared to CD32-CD4+ T cells. No HIV DNA was detected in CD32+HLADR- CD4+ T cells. Accordingly, HIV DNA was only slightly enriched (≤1.5 fold) in CD4+CD32+ pull downs relative to controls; and we observed a partial but not exclusive inducible p24 signal with PMA-induced CD4+CD32+ cells when compared to CD4+CD32- cells.

Conclusions: Our data highlight that CD32 may be preferentially expressed on activated CD4+ T cells harboring a transcriptionally active HIV reservoir rather than restricted to the resting latent HIV reservoir.

PP 2.0

Targeted screens identify new chromatin regulators of HIV latency

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Background: HIV cure strategies must reactivate the entire viral reservoir over time. In vitro maximal stimulation of host pathways fails to fully reactivate all latent, replication competent proviruses. This may suggest a greater role for restriction via epigenetic pathways. Our understanding of chromatin regulation via histone post-translational modification and the chromatin remodeling proteins which write, read, and erase these marks has significantly advanced in recent years, and so we re-examined epigenetic restriction in the context of HIV latency.

Methods: Selected based on literature review, a targeted list of chromatin regulators was subjected to lentiviral vector-delivered shRNA knockdown or inhibition via published chemical probes/inhibitors in three Jurkat latency models expressing GFP upon HIV transcriptional reactivation (2D10, JLatA2, JLat6.3).shRNA knockdown and/or small molecule inhibition was also assessed in combination with SAHA and TNFα at suboptimal doses to identify synergistic effects.GFP expression was assayed via high throughput flow cytometry.

Results: Our targeted shRNA screen identified a set of chromatin remodelers not previously implicated in HIV latency, the Polycomb-like (PCL) proteins PHF1/19 and two histone demethylases, KDM2B and KDM5A, that are linked to polycomb complex members. GFP expression was increased upon knockdown both with shRNA alone and in combination with SAHA and TNFα. Additionally, our screens have also identified UNC3866 and UNC4976, antagonists of the Polycomb CBX methyl-lysine reader family, as latency reversal agents (LRAs) when used combination with SAHA (1.4-2-fold increase in GFP), however both inhibitors fail to act as single agents.

Conclusions: These results further reinforce a role for the Polycomb pathway of H3K27 methylation-mediated chromatin repression in the control of HIV latency. The PCL-proteins have recently been implicated in PRC2 recruitment and may represent a novel mechanism for targeting of PRC2 to the HIV LTR. We have also demonstrated the first direct evidence for a role of PRC1 in maintenance of HIV latency via targeting of the PRC1 CBX methyl-lysine readers and highlight the potential importance of combinatorial LRA treatments for cure strategies.

PP 2.1

QVOA coupled with digital p24 analysis enhances HIV reservoir quantification

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Background: Latently infected resting CD4+ T cells (rCD4+) contribute to HIV persistence in individuals receiving long term suppressive ART. The low frequency of latently infected cells among the rCD4+ population presents challenges in quantifying the latent reservoir, which is critical to evaluate HIV eradication strategies. Several assays have been developed to quantify HIV latency, however all present limitations; PCR-based assays overestimate the HIV reservoir, while QVOA has been shown to underestimate it. To support the development of effective HIV eradication therapeutics, there is an urgent need to develop a robust and precise assay that more accurately quantifies the frequency of infected cells carrying replication-competent HIV. To address this need, we modified QVOA to include a digital p24 endpoint that shortened assay duration and increased sensitivity.

Methods: rCD4+ cells were enriched from cryopreserved PBMC from 5 ART-suppressed, HIV+ individuals provided by the Reservoir Assay Validation and Evaluation Network (RAVEN).Standard QVOA was performed, with culture supernatant collection on days 8, 12, and 20. IUPM were calculated by assessing the frequency of p24 positive culture supernatants at each time point as determined by Quanterix Simoa digital p24 system or ELISA.

Results: The digital p24 endpoint readily detected HIV+ samples that were below the limit of detection by ELISA. Analysis revealed patterns of viral growth kinetics at p24 levels not detected by ELISA, supporting identification of replicating virus present in QVOA that are missed by standard approaches. While ELISA efficiently detected robustly replicating virus with greatest sensitivity at day 20, digital p24 enabled detection as early as day 8 with greatest sensitivity at day 12. Use of digital p24 coupled with QVOA reduced assay duration and increased the estimated size of the latent reservoir (~7-fold) compared to day 20 IUPM measured by ELISA.

Conclusions: Digital p24 permits early and sensitive detection of HIV outgrowth enabling the duration of the assay to be reduced by 8–12 days, coupled with an approximate one log increase in the calculated IUPM. These data support that digital p24 offers an approach to improve the sensitivity of QVOA in quantifying the HIV reservoir, a critical component for effective development of HIV eradication strategies.

PP 2.2

Activation of mature dendritic cells via PKC agonist induces HIV-1 reactivation of latently infected cells

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Background: Bryostatin-1 is a clinically approved protein kinase C agonist that has shown significant ex-vivo potency to revert HIV-1 latency. This drug also activates dendritic cells (DCs) and modulates their immune profile. Here we aim to evaluate the effect of bryostatin-1 on mature DCs, the predominant antigen presenting cells residing in the lymphoid tissue, where viral reservoirs most likely persists.

Methods: Latently infected J-Lat cells with a tagged GFP HIV-1 provirus were cultured alone or with monocyte-derived dendritic cells matured with lipopolysaccharide (mDCs) in the presence of increasing concentrations of bryostatin-1. Viral reactivation was measured by FACS. Cytokine secretion was quantified by Luminex immunoassay. Effect of detected recombinant cytokines was tested in J-Lat reactivation assays. Transwells were used to investigate the role of cell-to-cell contacts in cellular reactivation in the presence or absence of a monoclonal antibody that blocks TNF-α (infliximab). Statistical differences were assessed by an upaired t test.

Results: Bryostatin-1 added directly to J-Lat cells had only marginal effects on viral reactivation, but this reactivation was 22-fold higher in the presence of mDCs (P=0.0017). Cytokine quantification of supernatants from co-cultures of mDCs exposed to bryostatin-1 revealed the increased production of IL-6, IL-8 and TNF-α when compared to mock treated co-cultures. Addition of recombinant IL-6, IL-8, and TNF-α (individually or in combination) to J-Lat cells cultured alone showed only viral reactivation by TNF-α. Accordingly, this reactivation was blocked by infliximab. Using a transwell to separate both types of cells we further reduced J-Lat reactivation. This indicates that membrane bound TNF-α produced by mDCs exposed to bryostatin-1 is playing a more prominent role in viral reactivation than soluble TNF-α. Therefore, bryostatin-1 action on mDCs induces the production of TNF-α, which in turn leads to the bystander reactivation of HIV-1 on J-Lat cells.

Conclusions: The use of bryostatin-1 to induce TNF-α secretion by mDCs at the lymphoid tissues might prove as a valuable tool to purge viral reservoirs.

PP 2.3

Single-cell transcriptomics to evaluate HIV latency establishment in primary CD4 T cells

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Background: HIV eradication is hindered by the existence of latent HIV reservoirs in CD4 T cells. In this study, we used a primary cell model of HIV latency and the dual reporter virus, HIV DuoFluo (provided by E. Verdin, UCSF) to investigate latency establishment in tonsil-derived CD4 T cells.

Methods: Tonsil tissue was obtained from HIV-negative individuals during elective tonsillectomy for sleep apnea at University of Miami Hospital. Purified CD4 T cells from a single tonsil donor were activated with anti-CD3/CD28 beads for 3d prior to infection with HIV DuoFluo, containing GFP under HIV-LTR promoter and mKO2 under EF1a promoter. Live, uninfected (U, GFP-mKO2-), latent (L, GFP-mKO2+), and productive (P, GFP+mKO2+/-) infected single cells were sorted using the Sony SH800 instrument into 96 well plates for 3′ whole transcriptome amplification using BD Precise platform. 124 cells from each population (U, P, and L) were collected over 2 independent experiments. After molecular reads were counted and data sanitized to exclude low count transcripts, Limma and FDR correction were applied to determine significant differences in gene expression.

Results: Principal component analysis showed that U and L cells overlapped with 0 DEGs that were able to pass the FDR correction cutoff and a fold change of >1.5 while P showed 202 DEGs compared to U and 155 compared to L. 121 DEGs overlapped between the 2 comparisons representing genes involved in HIV replication. One example is reduced expression in P cells of the non-coding RNA *RN7SK* which forms the 7SK small nuclear ribonucleoprotein (snRNP) complex that inhibits early transcript elongation by sequestering P-TEFb, a known co-factor for HIV transcription. Interestingly, *FCGR2A*, the proposed marker of latent cells in blood was not detectable in sorted single cells suggesting differences between blood and LN markers of HIV latency.

Conclusions: Our methods using an established model of HIV latency and single cell transcriptomics 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 2.4

Quantitation of the CD4+ T cell and macrophage reservoirs in SIV-infected ART-suppressed macaques: two functional latent reservoirs

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Background: HIV CURE requires an understanding ofcellular and anatomical sites harboring virus that would contribute to viral rebound upon treatment interruption. Despite ART, CD4+ lymphocytes are known to constitute a functional reservoir in blood and lymph nodes of HIV infected individuals. However, number of resting CD4+ T cells in tissues that contribute to the reservoir is unclear. During HIV and SIV infection tissue macrophage are infected and contribute to tissue specific diseases. Identifying macrophages and CD4+ T cell reservoirs in tissues and understanding their impact on the functional viral reservoir is critical.

Methods: Using a simian immunodeficiency virus (SIV)/macaque model for HIV AIDS, we evaluated whether infected cells persist in tissues despite ART. Seven SIV-infected pig-tailed macaques were virally suppressed with ART, and plasma and CSF virus loads were analyzed longitudinally. To assess whether virus persisted in tissue in these macaques, we used a macrophage and CD4+ T cell quantitative viral outgrowth assay (MΦ-QVOA and CD4-QVOA), qPCR and the levels of viral RNA and DNA in tissues.

Results: Viral RNA in tissues of suppressed macaques was undetectable although viral DNA was detected in all animals. The MΦ-QVOA demonstrated that the majority of suppressed animals contained latently infected macrophages in lung, spleen and brain. The comparison of latently infected CD4+ T cells and monocytes/macrophages in blood and spleen demonstrated a comparable level of functional latency in the two cells (~1 cell per million). The virus produced in the MΦ-QVOAs was demonstrated to be replication competent, suggesting that latently infected tissue macrophages are capable of reestablishing productive infection upon treatment interruption.

Conclusions: These studies provide the first comparison of CD4+T cell and monocyte/macrophage functional latency in the SIV ART suppressed macaque. It is also the first confirmation of replication-competent SIV in macrophages in tissues of ART-suppressed macaques. Our results suggest that the highly debated issue of viral latency in macrophages has been addressed in SIV-infected macaques treated with ART.

PP 2.5

Lack of transcriptional latency in infected primary cells in the presence of exosomes and cART

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Background: HIV-1 infection results in a chronic illness since long-term HAART can lower viral titers to an undetectable level.However, discontinuation of therapy rapidly increases virus burden.Moreover, patients under HAART frequently develop various metabolic disorders, neurocognitive abnormalities and cardiovascular diseases.

Methods: We routinely use a combination or ultracentrifugation and nanoparticle capture to concentrate our EVs from various bodily fluids for downstream assays including proteomics, Elisa, PCR, enzymatic extracellular vesicles and functional assays.

Results: In this study, we asked whether exosomes from uninfected cells could activate latent HIV-1 in infected cells. We observed that irrespective of cART, both short- and long-length viral transcripts were increased in infected cells exposed to exosomes. A search for a possible mechanism for this finding revealed that the exosomes increase RNA Polymerase II loading in infected cells. These viral transcripts, which include trans-activation response (TAR) RNA and a novel RNA we termed TAR-gag, can then be packaged into exosomes and potentially be exported to neighboring uninfected cells, leading to increased cellular activation.Also, recently we have found that several FDA-approved drugs have the ability to alter the content of exosomes released from HIV-1 infected cells. Furthermore, we will discuss how these findings on cART-altered exosomal content can be applied to general viral inhibitors (i.e. interferons) which are normally used in the treatment of HIV-1 and many other infections both in vitro and in vivo.

Conclusions: Collectively, these results imply that exosomes from uninfected cells activate latent HIV-1 in infected cells and that true transcriptional latency may not be possible in vivo, especially in the presence of cART. Also, our data implies that when patients are under antiretroviral therapy, they still release exosomes which may cause cytokine storm associated with neurocognitive and immunological dysfunction.

PP 2.6

DNA-PK regulates HIV transcription and latency by supporting the activity of RNA polymerase II and the recruitment of transcription machinery at HIV LTR

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Background: The regulation of HIV transcription plays a critical role in HIV life cycle, including proviral latency and reactivation. Despite the use of highly effective antiretroviral therapy (HAART), the presence of latent or transcriptionally silent proviruses prevents cure and eradication of HIV infection. These transcriptionally silent proviruses are well protected from both the immune system and HAART regimens. Thus, in order to tackle the problem of latent HIV reservoirs, it is a prerequisite to define all the pathways that regulate HIV transcription.

Methods: The data was obtained and confirmed using different methodologies, including Western blot, Chromatin immunoprecipitation (ChIP) assay and other molecular biology techniques.

Results: We have previously published that DNA-PK facilitates HIV transcription by interacting with the RNA polymerase II (RNAP II) complex recruited at HIV LTR. To extend those studies further here we demonstrate that DNA-PK promotes HIV transcription by facilitating transcription at multiple stages, which include initiation, elongation and RNAPII pause-release. DNA-PK-mediated increase to HIV transcription subsequently induces HIV replication and reactivation of latent HIV provirus. We found DNA-PK enhances HIV transcription by enhancing the phosphorylation of C-terminal domain (CTD) of RNAP II. CTD phosphorylation is required for the RNAPII become processive or elongation-proficient. We found DNA-PK augments CTD phosphorylation both by directly catalyzing and via recruiting P-TEFb at HIV LTR. Accordingly, the inhibition of DNA-PK via highly specific small molecular inhibitors profoundly restricts HIV transcription, replication and reactivation of latent provirus. To provide further physiological relevance, the results were confirmed in the peripheral blood mononuclear cells (PBMCs) isolated from HIV patients. Treatment of the cells with DNA-PK inhibitors resulted in severe impairment of RNAP II carboxyl-terminal domain (CTD) phosphorylation and establishment of transcriptionally repressive heterochromatin structures at HIV LTR.

Conclusions: Our results reveal the important role of DNA-PK in supporting HIV transcription, replication and latent proviral reactivation. Intriguingly, this study sheds light on an important pathway that affects HIV gene expression. These findings provide strong rationale for developing and using transcriptional inhibitors, such as DNA-PK inhibitors, as supplement to HAART regimens in order to further enhance their effectiveness and to suppress toxicity due to HIV proteins.

PP 2.7

Using barcoded HIV-1 to understand inducibility of the latent HIV-1 reservoir

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Background: The presence of a long-lived HIV-1 latent reservoir prevents patients from discontinuing antiretroviral therapy (ART). As ART is not curative, several strategies have been proposed to eradicate HIV. The "shock and kill" strategy is one such strategy to eliminate latent HIV-1 by inducing transcriptional reactivation of latent provirus with latency reversing agents (LRA) and targeting of cells that harbor reactivated HIV-1 with immune mediated mechanisms. However, LRAs do not appear to reactivate all replication-competent proviruses suggesting the presence of non-inducible proviruses. The presence of this non-inducible replication reservoir would inhibit strategies like "shock and kill" from eliminating the latent HIV-1 reservoir and thereby prevent patients from stopping ART. In order to eliminate the latent HIV-1 reservoir, it is necessary to gain further understanding of how latency occurs and what causes some latent proviruses to be non-inducible. We hypothesize that provirus integration site affects the inducibility of latent HIV-1.

Methods: In order to address our hypothesis we are synthesizing a barcoded HIV-1 library. A barcode consisting of a randomized 21-base-pair (bp) sequence was inserted in a lab-adapted strain of HIV-1, NL4-3, at three individual locations: between envelope and nef; the long terminal repeat (LTR) between R and U5; the LTR 15 bp from the end of U5. Upon inserting the barcode into the NL4-3 viral genome, the DNA library will be electrotransformed and individual colonies generated. SupT1 cells will be infected with viruses containing unique barcodes at each of the three insertion sites to evaluate the effect of inserting a barcode in each location on the biological activity of NL4-3.

Results: Forthcoming results will demonstrate the biological activity of NL4-3 containing unique barcodes at the three separate insertion sites.

Conclusions: Upon identifying where in NL4-3 a barcode can be inserted without affecting HIV-1's biological activity, a large HIV-1 barcode library will be generated containing a randomized 21 bp non-coding sequence. This HIV-1 barcode library will then be used to further understand HIV latency in an HIV-1 primary cell latency model.

PP 2.8

Increased expression and phosphorylation of SAMHD1 in SIV and HIV encephalitis is associated with proliferation of brain macrophages

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Background: HIV-1 infection of the brain and related cognitive impairment remain prevalent in HIV-1-infected subjects despite combination antiretroviral therapy. Sterile alpha motif and histidine-aspartate domain-containing protein 1 (SAMHD1) is a newly identified host restriction factor that blocks the replication of HIV-1 and other retroviruses in myeloid cells. Cell-cycle-regulated phosphorylation at residue Thr592 and viral protein X (Vpx)-mediated degradation of SAMHD1 have been shown to bypass SAMHD1 restriction in vitro. Herein, we investigated expression and phosphorylation of SAMHD1 in vivo in relation to macrophage infection and proliferation during the neuropathogenesis of HIV-1 and simian immunodeficiency virus (SIV) encephalitis.

Methods: Using brain and other tissues from uninfected and SIV-infected macaques with or without encephalitis, we performed immunohistochemistry, multi-label fluorescence microscopy, and western blot to examine the expression, localization and phosphorylation of SAMHD1.

Results: The number of SAMHD1+ nuclei increased in encephalitic brains despite the presence of Vpx. Many of these cells were perivascular macrophages, although subsets of SAMHD1+ microglia and endothelial cells were also observed. The SAMHD1+ macrophages were shown to be both productively infected and proliferating. Moreover, the presence of cycling SAMHD1+ brain macrophages was confirmed in the tissue of HIV-1-infected patients with encephalitis. Finally, western blot analysis of brain protein extracts from SIV-infected macaques showed that SAMHD1 protein exists in the brain mainly as an inactive Thr592-phosphorylated form.

Conclusions: The ability of SAMHD1 to act as a restriction factor for SIV/HIV in the brain is likely bypassed in proliferating brain macrophages through the phosphorylation-mediated inactivation, not Vpx-mediated degradation of SAMHD1.

PP 2.9

T-cell signaling pathways leading to reactivation of P-TEFb and HIV transcription elongation in resting memory T cells

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Background: Design of an effective combinatorial latency reversal regimen will require a detailed understanding of the host and viral mechanisms that are utilized by HIV to emerge from transcriptional latency. A complex series of molecular events in CD4+ T cells regulate

the availability of P-TEFb for control of latent proviral transcription. In resting memory T cells, P-TEFb is unassembled and therefore dissociated from the 7SK snRNP complex used to deliver it to genes. The extent of formation of functional P-TEFb strictly correlates with the efficiency of latency reversal and thus may be regarded as the central event required for the reversal of HIV latency. T-cell receptor stimulation of memory CD4+ T cells efficiently induces the formation of a functional 7SK snRNP complex containing P-TEFb through multiple complementary signaling pathways.

Methods: These events can be traced in real time by flow cytometry and immunofluoresence microscopy using antibodies that recognize specific post-translational modifications of P-TEFb.

Results: Nuclear localization of CDK9 kinase is coupled with its phosphorylation at Ser175, a marker of activated P-TEFb that we previously found to be important in allowing viral Tat to outcompete Brd4 for P-TEFb binding. Selective inhibition of Hsp90, CDK7, PI3K, mTORC1 and mTORC2 significantly suppressed the activation of P-TEFb in primary CD4+ T cells, as assessed by immunofluorescence staining for phospho-Ser175. These inhibitors also disrupted the biogenesis of P-TEFb by significantly reducing Cyclin T1 (CycT1) expression and preventing the phosphorylation of CDK9 on its T-loop at Thr186. Inhibition of Hsp90 or CDK7 kinase strongly suppressed the reactivation of latent HIV upon T-cell receptor stimulation in primary Th17 cells. Mutagenesis studies and molecular dynamics simulations based on published X-ray structures revealed that an intramolecular hydrogen bonding coordination of phospho-Thr186 by a conserved arginine triad is critical for the assembly of catalytically competent P-TEFb. Mutation of the arginine residues not only disrupted CDK9/CycT1 heterodimerization but also caused loss of phospho-Thr186 without affecting CDK9 interactions with the Hsp90/Cdc37 chaperone complex.

Conclusions: Our studies focus attention on the kinases that regulate CDK9 T-loop modifications required for the biogenesis of P-TEFb and which mediate intermolecular interactions with HIV Tat for proviral gene induction.

PP 2.10

The genetic traits of full-length HIV sequenced from memory T cell subsets

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Background: A thorough understanding of the distribution and genetic traits of replication-competent virus will be needed to design HIV eradication therapies. To address this, we used the Full-Length Individual Proviral Sequencing (FLIPS) assay to examine the contribution of genetically identical and intact proviruses within memory CD4+ T cell subsets to the latent reservoir during prolonged ART.

Methods: Naïve, central (CM), transitional (TM) and effector (EM) memory CD4+ T cells, as well as CD45RA-HLA-DR+ and CD45RA-HLA-DR- CD4+ T cells were sorted from the peripheral blood of six participants who initiated ART during either acute or chronic infection (n=3 each). Genetic sequences of HIV proviruses from the cell subsets were obtained using the FLIPS assay. FLIPS uses LTR-specific primers to amplify HIV proviruses at limiting dilution followed by next-generation sequencing. Proviruses were characterized as defective (containing INDELs, stop codons or hypermutation) or intact. Expansions of identical sequences (EIS) were determined as ≥2 identical HIV DNA sequences

Results: Of the 728 sequences isolated, only 5% were considered intact. Intact provirus was found in all cell subsets except CM (0/125 sequences intact). The proportion of intact provirus was different across the cell subsets (EM>TM>CM and HLA-DR+>HLA-DR-; p=0.001). The frequency of cells infected with intact proviruses was higher in HLA-DR+ memory T cells (48 vs <10 infected cells/million cells in HLA-DR+ vs all other subsets). Co-receptor usage was restricted, with 83% of intact proviruses CCR5-tropic. The percentage of intact and defective sequences contributing to an EIS was 34% (12/35) and 46% (319/693) respectively. In one participant 56 identical sequences contained a deletion in the packaging signal but were otherwise genetically intact. Despite this defect, the corresponding intracellular RNA sequence was detected.

Conclusions: Genetically intact and therefore likely replication-competent CCR5-tropic HIV is enriched in cells expressing HLA-DR and EM cells. This indicates that the latent HIV reservoir is established early and is maintained by cell proliferation and differentiation. The lack of intact virus in the CM suggests that the majority of rebound virus will not be derived from these cells. Defective proviruses can produce viral transcripts indicating that RNA quantification will lead to overestimating the genetically intact HIV reservoir.

PP 2.11

SIV proviral landscape differs from that of HIV-1 and shows gross hypermutation

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Background: HIV-1 establishes latency in a small pool of resting memory CD4+ T cells that represent the major barrier to cure. Various cure strategies are being tested in SIV-infected rhesus macaques as a model for HIV-1 infection. While the proviral genomic landscape has been characterized in HIV-1-infected patients, little is known about the proviruses persisting in SIV-infected macaques.

Methods: We developed an unbiased, single-genome-amplification method to capture both intact and defective proviral genomes from CD4+ T cells isolated from 7 long-term chronically SIV-infected ART-suppressed rhesus macaques. PCR primers were designed to amplify near-full-length proviral genomes. PCRs were set up at a limiting dilution, with one or fewer proviruses per well. Reactions were visualized on agarose gels and resulting bands were directly sequenced using either Sanger or Illumina platforms.

Results: At least 75% of proviruses were defective. Strikingly, over half of these proviruses showed gross hypermutation, including a subset that also contained internal deletions. Proviruses containing small, internal deletions at the 3′ end of the genome were also frequently detected. These deletions ranged in size and affected the env, tat, rev, and nef genes, while a distinct subset contained much larger deletions (>6 kb), encompassing most of the genome. In contrast to what was seen in CD4+ T cells isolated from HIV-1-infected patients on ART, a significantly larger proportion of proviruses had intact genomes. These findings differ from the proviral landscape of HIV-1-infected individuals who began ART during chronic infection, in whom 80% of proviruses had internal deletions, 7% were hypermutated, and 8% had both hypermutations and deletions.

Conclusions: The proviral landscape in these SIV-infected macaques is strikingly different than that in HIV-1-infected CD4+ T cells. A marked majority of SIV proviruses were grossly hypermutated. The pattern of hypermutation differs from HIV-1 proviruses in its severity, both across and within proviral genomes. Additionally, far more intact proviruses were detected in SIV-infected, ART-suppressed macaques than in HIV-infected, ART-suppressed patients. Other populations of SIV-infected monkeys will need to be studied, as these major differences between the HIV-1 and SIV pools of latent proviruses have implications for NHP models of HIV latency and treatment.

Session 3: *In vitro* and animal model studies of HIV persistence

OP 3.0

Testing cure approaches in NHPs: the Emory experience

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The availability of potent anti-retroviral drugs (collectively termed anti-retroviral therapy, ART) has resulted in a dramatic reduction in the the mortality and morbidity associated with HIV infection. However, no therapeutic strategy that can eradicate or functionally "cure" the infection has yet been developed. Our current inability to cure HIV infection is related to the presence of a persistent reservoir of latently infected cells that is resistant to both conventional anti-retroviral therapy (which targets specific phases of the "productive" virus life cycle only with no effect on the latent infection) and immune-based interventions (which require expression of viral proteins as target antigens for either cellular or antibody responses).

Over the past few years, the non-human primate models of SIV and SHIV infection of rhesus macaques have been validated for studies of virus eradication in the setting of fully suppressive ART. In this presentation, I will briefly review the Emory/Yerkes experience in studies of HIV eradication using the rhesus macaque model, including: (i) the opportunities presented by the SIV and SHIV macaque models to conduct pre-clinical studies aimed at developing and testing novel interventions aimed at achieving a functional cure for HIV infection; (ii) the main immune-based strategies that are currently explored to reduce or eliminate the virus reservoir in the NHP model; and (iii) the published and ongoing preclinical trials of immune-based interventions that are conducted by our team in ART-treated SIV-infected rhesus macaques with the goal of inducing a functional cure. Immune interventions that will be discussed include immune-based latency reversing agents (i.e. IL-15 agonists, CD8 depletion), type I interferons, interleukin-21, anti-IL-10 antibodies, FTY720, check-point blockade inhibition (i.e., inhibitors of PD-1, CTLA4, and LAG-3), agents that promote CD4+ T cell differentiation (i.e., beta-catenin and NOTCH inhibitors), and autologous stem cell transplantation.

OP 3.1

Visualization and quantification of HIV dissemination and reservoirs using *in vivo* imaging

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Background: Despite the importance of viral rebound, it is still unclear that why and how rebound occurs if antiretroviral therapy (ART) is removed. In vivo imaging has been used in daily clinical applications for diagnosing longitudinal changes deep in the tissues and organs. It mainly visualizes cells residing in tissues rather than fast moving blood cells, thus in vivo imaging is an excellent tool for visualizing HIV tissue reservoirs and rebound after ART.

Methods: Replication-competent HIV-NL-BaL was engineered to carry a biolumenescence reporter, such as firefly luciferase, eLuc, or deep sea shrimp luciferase, NanoLuc, without affecting its replication ability. It was inoculated in humanized Bone Marrow-Liver-thymus (BLT) mice via intravaginal (IVa.) or intraperitoneal (IP) inoculation, respectively. The infected cells expressing imaging reporter can be visualized for tracking HIV dissemination using bioluminescence imaging (BLI). Blood samples were collected for viral load and CD4/CD8 ratio for measuring HIV infection to be compared to the infceted tissue-resident cells.

Results: HIV infection in vaginal mucosa could be visualized as early as 2 days post inoculation at the vagina and replicated locally in the vaginal canal for at least 21 days before being visualized in human thymic orgonoid at dorsal side before disseminating robustly to other locations. In contrast, HIV-1 inoculated via IP injection could disseminated readily to human thymic organoid without any delay. After 2 wks of antiretroviral therapy, a complete suppression of HIV-1 infection was visualized. Only 25 days after ART withdrawal, the onset sites of viremia rebound were visualized.

Conclusions: 1) The imaging results of HIV-1 infection in BLT mice reveal significantly different dissemination pathways, mucosa vs. IP.

2) Suppression of HIV-1 replication in real time was visualized as early as in the first week of ART and forward.

3) HIV-1 rebound can be visualized 25 days after ART withdrawal for its initation site.

OP 3.2

Enhancing infection-resistant cells for HIV cure in the non-human primate model

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Background: Long-term protection of infection-susceptible cells is likely essential for HIV eradication. However, a substantial body of evidence suggests that actively targeting persistent HIV reservoirs, preferably under the cover of combination antiretroviral therapy (cART), may be an important tandem approach. We have developed a nonhuman primate model of HIV persistence and gene therapy to optimize protective strategies such as CCR5 editing and viral fusion inhibitors, and combined them with active targeting using CD4-based Chimeric Antigen Receptors (CD4CAR). We are interested in identifying the most promising and clinically relevant combinatorial therapies to induce cART-independent virus remission.

Methods: Macaques are infected with simian/human immunodeficiency virus (SHIV) containing an HIV envelope, and suppressed by cART. Following stable suppression, autologous hematopoietic stem and progenitor cells (HSPCs) are CCR5-edited, modified with lentiviral vectors expressing the peptide fusion inhibitor mC46, and/or engineered to express HIV/SHIV-specific CD4CAR. Gene therapy techniques are optimized in vitro and in mouse models. Virus persistence is quantified in cell-associated SHIV DNA and RNA, DNAscope and RNAscope, and quantitative viral outgrowth assays.

Results: The negative effects of broadly cytotoxic conditioning regimens, such as myeloablative irradiation, outweigh their impact on reservoir size, reinforcing the need for genetic protection strategies and safer conditioning. Transplantation with CCR5-edited HSPCs leads to significant decreases in tissue-associated SHIV DNA and RNA levels, although in situ analyses clearly demonstrate that persistent viral reservoirs remain. CD4CAR-modified HSPCs persist for years following transplantation, and are associated with decreased tissue-associated SHIV RNA levels and higher CD4:CD8 ratios in the gut. The number and function of these cells is proportional to the level of virus antigen.

Conclusions: HSPCs can be gene-modified and rendered HIV/SHIV-resistant, but must persist in higher numbers in vivo in order to impact persistent infection. Combination approaches such as mC46 + CD4CAR engender a potent source of infection-resistant, virus-specific immune cells. Applying these strategies in the setting of reduced intensity conditioning will enable more effective reservoir targeting with substantially less toxicity. Most importantly, HSPC-based therapies

provide a life-long source of "reservoir sentinels" that will augment the host response to recrudescent viral replication.

OP 3.3

Modeling the graft-versus-viral-reservoir effect in a nonhuman primate model of HIV persistence

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Background: Hematopoietic Stem and Progenitor Cell (HSPC) transplantation underlies the only known case of HIV Cure. Three facets thought to contribute to virus eradication are i) the myeloablative conditioning regimen, ii) transplantation with HIV-resistant CCR5Δ32 cells, and iii) an allogeneic graft-versus-host effect known as graft-versus-viral-reservoir (GVVR). We have previously shown that transplantation with autologous, unmodified hematopoietic stem cells is insufficient to eradicate the viral reservoir in a nonhuman primate (NHP) model. To dissect the impact of GVVR and HIV resistant cells, we have developed the first NHP model of allogeneic bone marrow transplant in Simian/Human Immunodeficiency Virus (SHIV)-infected, combined antiretroviral therapy (cART)-treated rhesus macaques.

Methods: Animals were infected with SHIV-1157ipd3N4 for up to six months, suppressed by cART for at least six months, then received haplo-identical and/or MHC-matched HSPCs following myeloablative total body irradiation with concurrent cART. Control animals did not undergo transplant. Some animals' HSPCs were transduced with lentiviral vectors expressing the peptide inhibitor of virus fusion, mC46, to generate infection-resistant donor cells. Donor/host chimerism and viral DNA/RNA levels were measured by PCR in select tissues longitudinally, and in >20 tissues at necropsy.

Results: Following infection, cART, and transplantation, animals were euthanized while stably suppressed. Analysis of whole blood and gated CD4+ T cells showed acquisition of 100% donor chimerism in peripheral blood at day 29 post-transplant, with lower T cell chimerism in 1 animal. Despite undetectable SHIV plasma viral loads post-transplant, viral reservoirs persisted in multiple tissues, including lymphoid and hematopoietic organs, gut, and CNS.

Conclusions: Consistent with previous clinical studies and our findings in the autologous setting, these results suggest that allogeneic transplantation alone cannot induce cART-independent viral remission. Reservoir eradication will likely require additional HIV resistance factors and strategies to augment virus-specific immunity, e.g. those guided by our studies of the mechanisms of GVVR. This combination therapy promises to protect donor cells against infection, and enhance the targeted killing of infected cells.

OP 3.4

Patient-derived HIV reservoirs can be stably engrafted into NSG mice and reactivated by latency-reversing agents *in vivo*

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Background: The inability of murine cells to support HIV replication has led to the development of 'humanized mouse models', comprising immunodeficient mice engrafted with human immune cells. Fatal graft-versus-host disease (GvHD) limits the utility of existing models for cure studies, however, by precluding the generation of fully quiescent reservoirs through long-term antiretroviral (ARV) suppression. We sought to circumvent this issue by engrafting NSG mice with $CD4^+$ T-cells from ARV-treated HIV-infected participants (pre-established HIV reservoir xenografts). We term this the CD4 ARV-treated mouse ($_{CAT}$mouse) model. We further tested whether engraftment of memory vs. total $CD4^+$ T-cells would delay the onset of GvHD.

Methods: NSG mice were injected with 10–50×106 memory or total CD4+ T-cells. Some subgroups were virologically suppressed by daily subcutaneous injections of ARVs (PMPA, FTC, DTG). GvHD was assessed by weight loss and physical appearance. CD4+ T-cell counts and phenotypes were monitored by flow cytometry. Plasma viral loads were assessed by qRT-PCR.

Results: In mice injected with total $CD4^+$ T-cells, we observed robust engraftment and the consistent onset of GvHD. This was accompanied by rapid viral rebound from patient reservoirs in 16/17 mice that had been previously treated with ARVs, and in 4/4 untreated mice. In contrast, the majority of animals that had been injected with memory $CD4^+$ T-cells remained free of signs of GvHD out to at least 26 weeks post-engraftment. Spontaneous viral rebound was less frequent in mice engrafted with memory $CD4^+$ T-cells, occurring in only 5/24 animals that were not treated with ARVs, and in 10/30 mice that had been treated with ARVs for 10 weeks following treatment interruption. Bryostatin treatment of memory $CD4^+$ $_{CAT}$mice induced *de novo* viral rebound in 7/19 previously ARV-treated mice and in 5/19 untreated mice at 14 and 9 weeks post-engraftment, respectively.

Conclusions: The engraftment of NSG mice with memory $CD4^+$ T-cells from ARV-treated participants holds promise as a model for testing *in vivo* latency reversal. Quiescent reservoirs were stable and maintained over 10 weeks of suppressive ARV therapy as evidenced by subsequent spontaneous and bryostatin-induced viral rebound following ARV interruption. Future directions include repeated experiments with more participants, and testing additional LRAs.

OP 3.5

SIV persists in lymphoid tissues despite alemtuzumab-induced CD4+ T cell depletion

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Background: Alemtuzumab (ATM) is a lymphocyte-depleting humanized anti-CD52 monoclonal antibody used for the treatment of multiple malignancies and licensed for relapsing-remitting multiple sclerosis. Here, we evaluated whether ATM can deplete long-lived latently infected CD4+ memory T cells in SIV-infected rhesus macaques (RM) on suppressive combination antiretroviral therapy (cART). We hypothesized that ATM-induced depletion and reconstitution in the presence of cART may significantly reduce SIV persistence.

Methods: Nine RM were intravenously inoculated with SIVmac239 and after 12 days received cART (tenofovir/emtricitabine/dolutegravir). Once sustained virus suppression (<15 RNA copies/ml) was achieved, 6 RM received intravenous doses of ATM at 5mg/Kg on days 0, 7, 14 and 29. Three RM did not receive ATM (controls). Lymph node (LN) and gastrointestinal tract (GIT) tissue was collected at 10 and 20 weeks. SIV DNA and RNA were quantified by qPCR/qRT-PCR and markers of immune activation by flow cytometry.

Results: ATM induced rapid depletion of circulating T cells, NK cells, B cells and monocytes, including a significant depletion in CD4+ memory and naive T cells in the blood (>95%) and some depletion

in LN (~50%). CD4+ T regulatory cells were also depleted (>90%). T cell reconstitution was associated with massive memory T cell proliferation as measured by Ki67, which peaked around 3–4 weeks post-ATM and followed by a gradual recovery of T cells in blood. After 7 months, naïve, central, and effector memory CD4+ T cells were 51%, 60% and 100% of pre-ATM levels, respectively. Post-ATM, but while still on cART, plasma SIV RNA remained detectable but below 15 copies/ml, with only 1 of 6 RM showing blips above 100 SIV RNA copies/ml. At 10 weeks post-ATM, total SIV DNA in peripheral blood mononuclear cells (PBMC) decreased from a mean of 2.4 to 1.4 log copies per 10^6 cells (p=0.03); however, SIV DNA in peripheral LN and GIT remained unchanged.

Conclusions: Although ATM can significantly reduce SIV DNA levels in the PBMC of SIV-infected RM on cART, low level viremia persists. The minimal effect on SIV DNA in LN and GIT suggests either limited depletion in those tissues or a rapid reconstitution at those sites post-ATM, perhaps by expanded clones.

OP 3.6

Differential viral rebound between lymph node and colon after treatment interruption in SHIV-infected rhesus macaques

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Background: Understanding the source of viral recrudescence after ART interruption will help target strategies for HIV cure.

Methods: Five Indian-origin rhesus macaques were inoculated with a single dose of SHIV1157ipd3N4 intrarectally at week 0. Daily ART (tenofovir/ emtricitabine/ dolutegravir) was administered from weeks 2 to 18. Peripheral blood, cerebral spinal fluid (CSF) and samples from the colon and inguinal lymph node were collected pre-infection, at weeks 2 (peak viremia), 18 (on ART), and when animals were euthanized 12 weeks after the detection of rebound viremia. SHIV-RNA was quantified by PCR. Infected cells in tissues were detected by RNAscope.

Results: Median peak plasma SHIV-RNA was 494,000 copies/mL. Plasma SHIV-RNA became undetectable 2 weeks after ART initiation and remained undetectable until week 18, when ART was stopped. Viral rebound occurred at a median (range) of 21 (17–28) days. Median peak viremia on rebound was 1890 (355-19900) copies/mL.

Median SHIV-RNA at peak was highest in colon, 24633 (470-115356) copies/mg, followed by lymph node, 2732 (119-17205) copies/mg then CSF 2050 (53-4720) copies/mL. SHIV-RNA was undetectable in all lymph node and colon samples on ART. However, after rebound, SHIV-RNA was detected in 3/5 animals (15-50813 copies/mg) in lymph node, in 1/5 animals (11 copies/mg) in colon and 0/5 animals in CSF.

While on ART, viral particles were seen in lymph nodes via RNAscope. After rebound, infected cells were detected in the colon in 1/5 and in lymph node in 3/5 animals.

Conclusions: Timing of rebound closely recapitulated acutely HIV-infected individuals. Despite higher viral burden in colon at peak viremia, viral rebound post ART occurred preferentially in lymph nodes on PCR and RNAscope. Inadequate ART penetration, relative inaccessibility of lymphoid follicles to HIV-specific CD8+ T-cells or disparity in target cell distribution during ART and rebound may potentially account for the presence of viral particles in lymph node despite suppressive ART.

PP 3.0

Persistence of SIV in the brain of SIV-infected Chinese rhesus macaques with or without antiretroviral therapy

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Background: Persistence of HIV-1 reservoirs in the central nervous system (CNS) is an obstacle to cure strategies. However, little is known about residual viral distribution, viral replication levels and genetic diversity in different brain regions of HIV-infected individuals on combination antiretroviral therapy (cART). Because myeloid cells particularly microglia are likely major reservoirs in the brain, and more microglia exist in white matter than gray matter in a human brain, we hypothesized the major viral reservoirs in the brain are the white matter reflected by higher levels of viral DNA.

Methods: To address the issue, we used the Chinese rhesus macaque (ChRM) model of SIV infection, and treated 11 SIVmac251 infected animals including long-term nonprogressors with cART for up to 24 weeks. SIV reservoirs were assessed by SIV DNA levels in 16 specific regions of the brain and 4 regions of spinal cord. Quantitative real-time PCR was used for meauring viral load levels in plasma, cerebrospinal fluid and cell-associted viral DNA in the CNS tissues. SIV env gp120 sequences were analyzed by phylogenetic trees. HIV Sequence Database Hypermut 2.0 program was used for analysis of frequencies of G-to-A mutations.

Results: We found relatively high frequencies of SIV in basal ganglia and brain stem compared to other regions. cART-receiving animals had significantly lower SIV DNA levels in the gray matter than white matter. Moreover, a shortened envelope gp120 with 21 nucleotide deletions and guanine-to-adenine hypermutations were observed.

Conclusions: These results demonstrate that SIV enters the CNS in SIV-infected ChRM with a major reservoir in the white matter after cART. The SIV/ChRM/cART is an appropriate model for studying HIV CNS reservoirs and testing new eradication strategies. Further, examining multiple regions of the CNS may be needed when assessing whether an agent is successful in reducing the size of SIV reservoirs in the CNS.

PP 3.1

Evaluation of the in vivo capacity of broadly neutralising anti-HIV antibodies to eliminate latently infected cells from HIV-infected individuals using a novel humanised mouse model

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Background: Persistent latent HIV-infected cells capable of producing infectious virus are the primary obstacle to curing HIV-1 infection. They are rare and difficult to target, making their elimination challenging. The development of treatments to cure HIV is hampered by the lack of latency models which recapitulate the in vivo activation and elimination of latent infected cells from patients.

Methods: We developed a novel pre-clinical model to evaluate in vivo strategies to eliminate reactivated primary latent infected cells (huPAT-LAT mice). Immunodeficient NSG mice were intrasplenically injected with PBMCs (30 x 10^6 cells) from a leukapheresed HIV-infected donor containing latent HIV-infected CD4+ T cells (~10 IUPM by QVOA), virally suppressed by cART (<50 copies/ml) for 8 years. To maximize latent cell-activation, we co-injected irradiated allogeneic cells. We investigated whether broadly neutralizing antibodies (bNAbs) could reduce the latent reservoir by mobilizing antibody dependent cellular cytotoxicity (ADCC). huPAT-LAT mice were treated with one dose of bNAb 10-1074 or 10-1074 whose Fc domain contains a GRLR mutation (10-1074-GRLR) to reduce FcgR binding and ADCC activity. We bled the huPAT-LAT mice weekly and measured plasma viremia by qPCR and 10-1074 levels by ELISA. After 5 weeks, spleens were harvested and viral DNA and RNA were quantified by qPCR.

Results: Viremia (average >10^5 copies/ml) was detected in all huPAT-LAT mice 1 week after PBMC injection, which persisted until sacrifice 4 weeks later. Viremia was completely suppressed in all of the 10-1074-treated hu-PAT-LAT mice for at least two weeks. Three weeks after 10-1074 injection, viremia was detected in some 10-1074-treated mice while other 10-1074-treated mice displayed further delay in the onset of the viremia and/or reduction in the amplitude of the viremia. huPat-Lat Mice treated with 10-1074-GRLR displayed more rapid onset of viremia than 10-1074-treated mice, supporting the role of Fc-mediated ADCC in reducing the viral reservoir, which was further bolstered by sequence analysis of plasma virus.

Conclusions: We developed a new pre-clinical humanized mouse model that enabled us to demonstrate that bNAb-mediated in vivo elimination of reactivated latent HIV-infected cells from an HIV-infected patient and can be used to evaluate the efficacy of other HIV cure treatments.

PP 3.2

Assessing antiretroviral drug (ARV) bioavailability in HIV tissue reservoirs using *in vitro* and *in vivo* pharmacokinetic (PK) studies with human primary lymphoid endothelial cells and mice

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Background: The secondary lymphoid tissues (LT), lymph nodes (LN) and gut-associated lymphoid tissue (GALT), and the brain are considered potential reservoirs for HIV. Antiretroviral drugs (ARVs) have lower penetration into LT. In vitro models predictive of ARV LT penetration have not been established. The objective of this work was to develop and evaluate human lymphoid endothelial cells (hLECs) as an in vitro model of LT bioavailability and investigate predictability with in vivo PK studies of ARVs in mice.

Methods: We established a hLEC model and evaluated bioavailability of 13 ARVs at maximum plasma concentration (Cmax) observed in HIV-1 infected patients. ARVs were: maraviroc (MVC), abacavir (ABC), emtricitabine (FTC), tenofovir disoproxil fumarate (TDF), efavirenz (EFV), rilpivirine (RPV), dolutegravir (DTG), elvitegravir (EVG), raltegravir (RAL), atazanavir (ATV), darunavir (DRV) and cobicistat (COBI) and ritonavir (RTV). ARVs with higher (EFV), intermediate (DTG) and lower (FTC) hLEC bioavailability were evaluated for bioavailability in LN, brain and gut tissues of BALB/c mice given 50/10/30 and 15/15/15 mg/Kg orally, daily for 3 days. Concordance of in vitro and in vivo bioavailability was examined.

Results: ARVs showed high (>67th percentile; RPV, EFV, EVG, COBI), intermediate (67th-33rd; RTV, TDF, DTG, MVC) and low (<33rd; ATV, DRV, RAL, FTC, ABC) bioavailability in hLECs.EFV, DTG and FTC bioavailability hierarchy in mice tissues was:LN, EFV > FTC > DTG; gut, FTC > EFV > DTG; brain, EFV > DTG > FTC. The hLEC studies predicted higher bioavailability of EFV than DTG and FTC in LN and this was observed in mice. In contrast, FTC showed higher LN bioavailability in mice than DTG.

Conclusions: An in vitro primary hLECs model was developed to investigate ARV penetration. ARVs displayed distinct patterns of hLECs penetration. PK studies in mice with EFV, DTG and FTC were concordant with prediction of EFV having highest LN bioavailability. Additional in vivo PK studies with other ARVs are needed to thoroughly determine predictability of the hLEC model for in vivo tissue bioavailability, as well as to provide leads to identify ARV characteristics and mechanisms essential for penetration in tissue reservoirs.

PP 3.4

Next generation viral outgrowth assays as proxies for classic QVOA to measure HIV-1 reservoir size

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Background: Evaluations of HIV curative interventions require efficient assays that reliably quantify the latent reservoir.The "classic" quantitative viral outgrowth assay (QVOA) is regarded as "gold standard," but is impractical for widespread use. We compared five induced outgrowth assays employing PCR or ultrasensitive p24 readout of short-term CD4+ co-cultures (next-gen assays) to assess as scalable proxies for QVOA.

Methods: Next-gen assays were compared to classic QVOA results on fresh and frozen leukapheresis samples from 5 ART-suppressed HIV+ participants and one HIV- control. We used Markov chain Monte Carlo methods to estimate extra-Poisson variation at the aliquot, batch, and lab levels.Models also estimated the effect of using frozen versus fresh samples.

Results: Next-gen assays had similar estimates of variation to QVOA, with random variation at aliquot, batch, and lab levels having overlapping credible intervals. Overall, RNA-based assays reported higher IUPM than classic p24-based assays. Assaying split samples in the same batch had 2.5-fold extra-Poisson variation (95% CI 2.1 – 3.5) for next-gen assays. Assay performance by two separate labs increased total extra-Poisson variation to 3.4-fold (95% CI 2.6 – 5.4). Frozen storage did not substantially alter IUPM (–18% (–52%, +39%)).Within this cohort, two of the next-gen assays using short-term stimulation and PCR or ultrasensitive p24 readout had moderately high correlation with all four classic assays (R2 > 0.5 for all four comparisons, R2 > 0.8 for at least one comparison).

Conclusions: The data offer cautious support for use of next gen assays as proxies for more laborious outgrowth-based QVOA, while providing greater sensitivities and dynamic ranges. Measurement of LR in eradication strategies would benefit high throughput and scalable assays.

PP 3.5

Broadly neutraliing antibody cocktail prevents the establishment of viral reservoir against a mixed SHIV challenge

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Background: HIV-1 diversity presents a major challenge for the clinical development of bNAbs. Our recent study has demonstrated that combination of anti-V3 PGT121 and anti-V2 PGDM1400 provides fully protection against a mixed challenge with clade B SHIV-SF162P3 and clade C SHIV-325C in NHP models, suggesting that combination therapy may be necessary for protective efficacy against HIV in people (Julg B*, Liu PT*, et al. Sci Transl Med 2017). However, the question of whether multiple bNAbs will be required to prevent the seeding of primarily sensitive virus in reservoirs has not been studied in detail.

Methods: 20 rhesus monkeys (N=5/group) were infused i.v. with PGDM1400, PGT121, the combination of both bNAbs, or PBS as control. Animals were challenged the following day by i.r. route with SHIV-SF162P3+SHIV-325C. PBMCs were harvested at week 6. Viral DNA were measured using either gag-targeted or strain-specific quantitative RT-PCR. To identify which virus(s) established infection, an unbiased, single-genome-amplification method was designed to target conserved region of SHIV-SF162P3 and -325C, following by Sanger sequencing.

Results: Viral DNA measured in PBMCs showed that animals received 325C alone and in control group has similar viral DNA loads (3.56 and 3.37 log copies/million cells). Animals that received PGT121 alone showed lower viral DNA loads (2.25 log copies/million cells). In contrast, none of the animals that received the combination of PGT121+PGDM1400 exhibited detectable viral DNA loads. Viral sequencing by single genome analysis indicated that 100% of clones in the PGT121-treated animals were SHIV-325C, whereas 100% of clones in the PGDM1400-treated animals were SHIV-SF162P3, consistent with the resistance patterns of these viruses. These findings were also confirmed by strain-specific RT-PCR assays.

Conclusions: These data reveal that single bNAbs can specifically select for resistant viral variants within a diverse challenge swarm, resulting in the establishment of reservoirs with resistant SHIV strains. These findings suggest that a combination of bNAbs will be required to provide optimal protection against acquisition of HIV-1 infection. Nevertheless, further analysis by deep sequencing will be required to identify the existence of primarily sensitive virus in the reservoirs under bNAbs therapy.

PP 3.6

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

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Background: A robust simian-human immunodeficiency virus (SHIV)-rhesus macaque (RM) model of HIV-1 latency is critical to investigate eradicative and suppressive strategies that engage Env.We have developed a novel strategy to generate designer SHIVs encoding native (transmitted/founder) TF or primary Envs with tier 2 neutralization that consistently confer productive infection, high peak viremia and desirable early viral kinetics.Here, we evaluate two promising TF SHIVs, SHIV.D.191859 and SHIV.C.CH848, which encode TF subtype D and C HIV-1 Envs, respectively, for their viral kinetics and persistence during suppressive combination antiretroviral therapy (cART) and treatment interruption in RM.

Methods: 12 Indian RM were intravenously or mucosally inoculated with SHIV.D.191859 and 8 RM were intraveneously inculated with SHIV.C.CH848; all were followed longitudinally. Viral kinetics through the establishment of peak and setpoint viremia, 24 weeks of cART, and treatment interruption were assessed via plasma RT-PCR.Single genome sequencing of plasma virus was used to characterize the diversity of rebounding viruses.

Results: Inoculation of 12 RM with SHIV.D.191859 led to productive infection with peak viral loads between 10^5–10^8 copies/ml.In 11 of 12 animals, viremia was maintained for at least 6 months. At between 6 and 18 months of infection, 4 RM with high setpoint viremia (viral load of 10^3–10^7 copies/mL) were placed on cART for 24 weeks.Viral suppression was rapidly achieved and durably maintained. Viral rebound between day 7 and 17 was observed in all four rhesus macaques upon treatment interruption. Sequencing of rebound plasma vRNA revealed multiple genetically distinct virus populations at first detectable rebound in all four animals. Inoculation of 8 RM with SHIV.C.CH848 produced desirable viral kinetics with peak viremia of 10^7–10^8 and set point viremia between 10^3–10^5 copies/ml. After 16 weeks of infection, the 4 RM were placed on cART and viral suppression was rapidly achieved and maintained for 24 weeks. Viral rebound occurred at day 12–29 after treatment interruption. In both SHIV.D and SHIV.C infected RM, time to rebound correlated with setpoint viremia.

Conclusions: The antigenic properties and viral kinetics before, during and upon interruption of cART make SHIV.D.191859 and SHIV.C.CH848 promising reagents for a SHIV model of HIV-1 latency and cure.

PP 3.7

HIV latency reversal using designed PKC modulators

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Background: HIV can persist for many years in a latent state in individuals treated with antiretroviral therapy (ART), and these latently-infected cells represent a major barrier to curing the infection. One potential approach for eliminating this latent reservoir is to induce the virus to express viral proteins, which would make the host cell susceptible to viral cytopathic effects, immune effector mechanisms, and other therapeutic approaches. For this strategy to be successful, safe and effective methods for activating latent HIV expression are needed.

Methods: Towards this goal, we have designed and synthesized new, potent HIV latency reversing compounds based on prostratin and bryostatin that function through protein kinase C (PKC). In the current study we aimed to test these for relevant bioactivity in vitro using various cell line latency models, ex vivo using latently-infected cells from ART-treated patients, and in vivo using both wild-type and humanized mice.

Results: Several of our designed, synthetic PKC modulating compounds were highly effective in reversing HIV from latency in cell line models and latently-infected cells obtained ex vivo from ART-treated patients. Promising compounds were then evaluated for acute toxicity and bioactivity in immunocompetent mice. In this assay prostratin and bryostatin analogs that were more bioactive and less toxic in immunocompetent mice than the corresponding natural products were identified. One particularly potent and well-tolerated bryostatin analog was then tested for its ability to reverse HIV from latency in HIV-infected humanized BLT mice treated with ART. This bryostatin analog was found to be capable of activating expression of latent HIV in peripheral blood and tissues following in vivo administration and caused some of the newly productively infected cells to die.

Conclusions: Results indicate that designed, synthetically-accessible, tunable, and efficacious bryostatin analogs can mediate both a "kick" and "kill" response in latently-infected cells and exhibit improved tolerability. This study therefore demonstrates how natural leads can be used as an effective template for the design of more accessible, efficacious, better tolerated and tunable analogs, representing promising adjuvants for use with ART in strategies to eradicate HIV. suppressive factors like CD8+ T-cells contribute to viral latency even on long-term ART.

PP 3.8

In vitro and *in vivo* quantification of HIV-induced neuroinflammation and effect of antiviral agents in primary human microglia and a murine HAND model

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Background: Persistent HIV infection in the CNS despite life-prolonging antiretroviral (ARV) treatment increases activation of macrophages (MΦ), microglia, and surrounding astrocytes/neurons, conferring HIV-induced inflammation resulting in HIV-associated neurocognitive disorders (HAND). We developed a novel primary human stem cell-derived microglia cell system to quantify effects HIV-induced neuroinflammation and persistence.Additionally we developed a high throughput FACS based readout to evaluate the potency of ARV and quantify brain pathology in a murine model of HAND.

Methods: Stem cell or monocyte-derived microglia were differentiated with GM-CSF, IL-34, dorsimorphin, fibrinogen-growth-factor-2, and neuro-broth medium.Cells were confirmed pure (FACS) by antibody staining with: CD11b+/CD3-/CD14-/CD3-/CD14-/CD45+/CD11b+/CX3CR1+/CD80/86-/IBA-1+/CD39(lo+). MΦ were differentiated with GM-CSF. Cells were infected with HIV-1BaL for 4 hr with various concentrations of anti-inflammatory Jak 1/2 inhibitor baricitinib, AZT or Darunavir followed by culture in drug-containing medium for 14 days prior to viral quantification (RT assay). HLA-DR/CD163 were quantified (flow cytometry). Human MΦ were infected with HIVADA and used in our established HIV-HAND model. Day 14 days post infection, mice were sacrificed; brains were removed and single-cell suspensions were stained (CD45, MAP2, GFAP, MHCII, human-CD163, or p24; flow cytometry).

Results: In microglia, all antiviral agents except AZT demonstrated significantly ($p < 0.01$) greater potency compared to MΦ. Baricitinib significantly reduced ($p < 0.01$) HIV-induced activation of microglia and MΦ (CD163/HLA-DR). In mice, GFAP/CD45/MHCII (astrogliosis and macrophage infiltrate markers) increased significantly ($p < 0.01$) in HIV-infected brains versus uninfected controls. MAP2+ (neuronal cells) were significantly ($p < 0.05$) reduced in HIV-infected brains versus uninfected brains.

Conclusions: We demonstrated for the first time that primary stem cell-derived microglia can be infected with HIV, and that this system can be used to quantify effect of antiviral agents on HIV replication and HIV-induced inflammatory events driving HAND.These data represent an innovative, physiolgically relevant primary cell system that can be used in tandem with our murine HAND model to evalulate agents targeting reservoirs and viral persistence in the CNS.

PP 3.9

Fully MHC-matched allogeneic hematopoietic stem cell transplantation in SIV-infected, cART-suppressed Mauritian cynomolgus macaques indicates GVHD as a reservoir clearance mechanism

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Background: Timothy Brown remains in cART-free HIV remission following allogeneic hematopoietic stem cell transplant (HSCT), but attempts to recapitulate his cure have been unsuccessful. We recently established a nonhuman primate model of fully MHC-matched allogeneic HSCT to investigate the mechanism of cure in Timothy Brown. In this model, a reduced intensity conditioning (RIC) regimen consisting of chemotherapy, CD3 depletion, and total body irradiation (TBI) prior to HSCT results in durable, full multi-lineage donor chimerism in SIV-naïve HSCT recipients. Here, we sought to investigate (1) if similar results could be achieved without TBI, and (2) the impact of allogeneic HSCT on SIV reservoir size in cART-suppressed, SIV-infected recipients.

Methods: Allogeneic HSCT was performed with fully MHC-matched Mauritian cynomolgus macaque (MCM) donor-recipient pairs, including two cART-suppressed, SIV-infected recipients. Mobilized peripheral stem cells collected from donors by leukapheresis were transplanted into recipients following RIC with or without TBI. Donor engraftment was monitored by Illumina sequencing of single nucleotide polymorphisms. Immune subset reconstitution was assessed longitudinally by flow cytometric phenotyping and complete blood counts.

Results: We performed two HSCTs without TBI, both resulting in low T cell donor chimerism (<15%). The first, SIV-naïve recipient experienced incomplete T cell rebound, polyoma virus reactivation, and euthanasia. The second, cART-suppressed, SIV-infected recipient stabilized post-HSCT, and experienced a reduction in lymph node-associated SIV DNA despite incomplete T cell donor chimerism. Adding back TBI for HSCT of a second cART-suppressed, SIV-infected recipient resulted in high levels of donor chimerism in whole blood (>95%) and T cells (~80%) within 30 days of HSCT. Subsequent donor lymphocyte infusion increased T cell donor chimerism levels to nearly 100%, but led to development of clinical graft-versus-host disease (GVHD) and euthanasia. At necropsy, SIV DNA was below the limit of detection in lymph nodes, constituting a ~3 log reduction from pre-HSCT levels.

Conclusions: These data demonstrate that TBI is critical to achieving high levels of T cell donor chimerism post-HSCT in MCM, and that while immune conditioning decreases the viral reservoir size, GVHD is associated with enhanced reservoir clearance. This model will facilitate future studies investigating mechanisms of HSCT-mediated HIV cure. I will discuss our latest results.

PP 3.10

Influence of sex as an intrinsic biological variable in a primary cell model of HIV latency

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Background: Several cohort studies have shown that there are sex differences in the pathogenesis of HIV-1. Recently, it has been shown that females are more likely to develop a lower reservoir size than male during long-term ART. The immunologic and virologic mechanisms underlying these clinically relevant observations are not currently understood. We wanted to address whether sex was an intrinsic difference in CD4 T cells that could explain some of the clinical observations.

Methods: We have used a primary cell model of HIV-1 latency that recapitulates the generation of latently infected cultured TCM and uses a replication competent virus and ART. Using this primary cell model, we have characterized whether sex influences the intrinsic ability of HIV to replicate and to establish latency in cultured TCM cells. Furthermore, we have characterized the ability of different Latency-Reversing Agents (LRAs) to reactivate latent HIV.

Results: We have found that cultured T_{CM} from both sexes can replicate HIV at similar ratio and generate equal levels of latent infection. Analysis of the ability of several LRAs to reactivate latent HIV suggest that sex is not an intrinsic biological variable to the activity of these specific LRAs.

Conclusions: Our results show that sex is not an intrinsic biological variable in CD4 T cells in terms of the ability of HIV to replicate, to establish latency or to reactivate from latency with several LRAs. Other extrinsic factors may be accountable for the sex differences observed clinically.

Session 4: Virology of HIV persistence

OP 4.0

Characterizing HIV expression of proviruses during ART in tissues and blood

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Background: HIV proviruses persist during ART in latent and transcriptionally-active forms. Little is known regarding the fraction of infected cells in blood and in tissues that are quiescent versus those that actively express unspliced HIV-1 RNA in either the total population of infected cells or in infected cell clones. The fraction of cells that actively transcribe HIV-1 RNA during ART may be associated with the time to viral rebound if ART is interrupted and/or to the rate of spontaneous reactivation of latently-infected cells.

Methods: PBMC and LNMC were obtained from five donors: four had <40 cps/ml on ART for 4.3–12.9 years and one was ART-naïve. Proviral populations and their expression were characterized by cell associated RNA- and DNA- single genome sequencing of p6-PR-RT to determine the fraction of infected cells with transcriptionally-silent proviruses and the fraction with proviruses that were undergoing transcription in vivo at the time of sample collection.

Results: The number of infected cells in the PBMC and LNMC was not different during full suppression on ART, especially when normalized for the total number of CD4+ T cells in each compartment (p=0.9). Transcriptionally-active proviruses, including those in probable cell clones, were found in both LNMC and PBMC. However, a greater fraction of infected cells in LNMC contained unspliced HIV RNA both before and during ART than in PBMC (mean 13% vs. 6%, p=0.01). High-expressing cells (>20 HIV RNA copies/cell) were observed in the untreated individual but not in samples collected on ART, with the exception of one LNMC.

Conclusions: Greater than 80% of HIV proviruses in vivo are transcriptionally-silent after long-term suppression of viremia on ART. However, a higher fraction of infected cells in the lymph nodes have transcriptionally-active proviruses during ART than in the peripheral blood. Profiling HIV proviral expression during ART will lead to a better understanding of the HIV reservoir, the nature of latency, and the sources of rebound viremia when ART is interrupted.

OP 4.1

HIV-1 mediated insertional activation of STAT5B and BACH2 promotes the formation of a viral reservoir in T regulatory cells

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Background: It has been shown that HIV-1 insertions targeting BACH2 and STAT5B are enriched and persist for decades in hematopoietic cells from patients under Anti-Retroviral Therapy (ART), suggesting that insertional mutagenesis could provide a selective advantage to these cell clones. However, the mechanisms used and the physiological impact on the cells harboring these integrations are completely unknown.

Methods: In the hematopoietic cells of 30/87 patients under ART we identified chimeric mRNA containing viral HIV-1 sequences fused to the first protein-coding exon of STAT5B or BACH2. By performing droplet digital PCR, we found that these chimeric mRNAs were specifically enriched ($p<0.001$) in T regulatory (Treg) cells in all patients tested (N=9). Forced expression of STAT5B and BACH2 in Treg cells purified from healthy donors did not alter their phenotype and functions in vitro and significantly increased their proliferative capacity in competitive proliferation assays ($p<0.0001$). Co-injection in immune-deficient mice of GFP-, BACH2- and STAT5B-transduced Treg cells with allogenic PBMCs prevented xenogeneic graft-versus-host disease in 75% of the animals and reduced the level of human chimerisms in the blood of mice receiving STAT5B-expressing Treg cells when compared to mice treated with GFP-expressing Treg cells ($p<0.001$), suggesting for a superior activity of STAT5B-expressing cells in controlling the expansion of human PBMC.

Results: These data provide evidences that HIV-1 takes advantage of insertional mutagenesis to favor its persistence in the host by infecting long-living and self-renewing cellular reservoir endowed with the ability to diminish the immune surveillance against infected cells.

Conclusions: New targeted therapies aimed at interfering with BACH2 and STAT5B pathways could be exploited to reduce cellular reservoirs and favor the eradication of the infection in cART-treated patients.

OP 4.2

Productive HIV-1 infection upregulates CD32 *in vitro* and *in vivo*

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Background: The cell surface receptor protein CD32a has recently been postulated as a marker of a CD4 T-cell HIV reservoir harboring replication-competent proviruses in ART-suppressed subjects. The expression of CD32 in different CD4+ T-cell subsets in HIV-infected patients and expression in persistent infected cells retained within lymphoid tissue after ART are important questions that still need to be evaluated

Methods: The expression of CD32 in different CD4+ T-cell subpopulations was measured in 9 ART-treated patients by fluorescence activated cell sorting (FACS) immunophenotyping using antibodies that recognized CD3, CD4, CD27, CD45RO, CD32 and HLA-DR. HIV and CD32a RNA expression in tissue was measured by fluorescent in situ hybridization (FISH) in lymph node sections from 2 aviremic and 4 viremic HIV-infected patients. Ex vivo HIV-1 infection kinetics was measured by the RNA/flow technique during 6 days after the initial infection of healthy donor unstimulated PBMCs, and in a cervico-vaginal tissue explant model of HIV infection.

Results: Terminally-differentiated CD4+ T cells expressed significantly higher proportions of CD32 receptor compared to other CD4+ T subpopulations ($p<0.05$ for all comparisons). The majority of CD4+CD32+ cells had a naïve phenotype (CD45RO−CD27+) followed by central memory (CD45RO+CD27+) and terminally-differentiated phenotype (CD45RO−CD27−). In vivo, the vast majority of productively infected cells in lymph node tissues from HIV

aviremic and viremic patients also co-expressed the CD32a marker (90% of all infected cells), while most of CD32a single positive cells were absent from the B cell follicle, one of the major reservoirs for HIV.HIV infection of unstimulated pbmcs and cervico-vaginal histocultures upregulated the expression of CD32 in approximately 10-20% of all infected cells. CD32+ infected cells expressed more frequently the activation marker HLA-DR compared to CD32- infected cells (92% vs 77%) and the immune check-point PD-1 (46% vs 25%).

Conclusions: CD32 is preferentially detected in HIV transcriptionally active cells in tissues after ART and was identified to be co-expressed with activation markers in in vitro infected cells suggesting its expression is also associate with active HIV-infected cells.

OP 4.3

No evidence for ongoing HIV replication in lymph nodes during suppressive ART

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Background: Lymph nodes have been implicated as potential sanctuary sites for HIV replication on ART due to insufficient drug penetration. To investigate this possibility, we characterized HIV proviral populations, their levels of expression, and their sites of integration in paired lymph node and peripheral blood mononuclear cells (LNMC and PBMC) collected on ART.

Methods: PBMC and LNMC were obtained from five donors: four had <40 cps/ml on ART for 4.3-12.9 years and one was ART-naïve. Pre-ART samples were obtained for three of the treated patients. Longitudinal on-ART LNMC from two different inguinal nodes were obtained from two patients one year apart. Proviral genetics and expression were characterized by cell associated RNA- and DNA- single genome sequencing of p6-PR-RT. Sequences were compared phylogenetically and by testing for panmixia. Infected cell clones were identified by integration sites assay (ISA) in PBMC and LNMC from one donor.

Results: Comparisons of the proviral sequences on ART in PBMC (n=176) and LNMC (n=234) showed no increase in branch length, diversity, or divergence from pre-ART plasma or PBMC due to ongoing viral replication in either location. A test for panmixia of proviral sequences in PBMC and LNMC (probability of panmixia>0.02; threshold at p<10-3 for multiple comparisons) and across two separate lymph nodes sampled contemporaneously (probability of panmixia>0.3) showed no evidence for compartmentalization. Transcriptionally active proviruses, including those in probable cell clones, were found in LNMC and PBMC, but a greater fraction of infected cells in LNMC was expressing HIV RNA than in PBMC (13% vs. 6%). The proviral expression levels in single cells was not different on-ART between PBMC and LNMC (p>0.09). In one patient, forty clones of infected cells were identified by ISA. There were no differences in the detection of these clones in PBMC vs. LNMC (p=0.8).

Conclusions: Comparison of proviral populations, including clones of infected cells, and their expression in LNMC and PBMC, showed that HIV populations were well-mixed. There was no evidence for divergence from pre-ART populations in PBMC or in LNMC, which is not consistent with the HIV reservoir being maintained by ongoing cycles of viral replication in either PBMC or LNMC during suppressive ART.

OP 4.4

Tissue macrophages are a major viral reservoir in male urethra of HIV-1-infected individuals under suppressive antiretroviral therapy

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Background: HIV-1 eradication requires the elimination/reduction of the HIV-1 reservoir pool. The best-characterized HIV-1 reservoir resides within T-cells. Yet, residual viremia in HIV-1-infected cART-suppressed individuals originates not only from T-cells but also from macrophages, which could therefore comprise an additional long-lived HIV-1 reservoir. Indeed, macrophages, an initial target of HIV-1 infection in the genital mucosa, are tissue-resident cells that resist the cytopathic effects of HIV-1 infection, are long-lived, can self-renew, accumulate infectious virus in intracellular virus-containing compartments (VCC), and can produce infectious virus upon stimulation in-vitro. Whether in cARTpatients, tissue-macrophages form HIV-1 reservoirs from which replication competent virus can be produced upon stimulation in vitro remains unclear.

Methods: Using whole penile tissues, obtained upon transgender surgery, from HIV-1-infected cART-suppressed individuals with undetectable plasma viral loads, we searched by PCR, FISH and microscopy for HIV-1 DNA, RNA, p24 and intact virions. Infectious reactivation competent virus was measuredin a sensitive quantitative viral outgrowth assay (sQVOA).

Results: We found that urethral macrophages contain HIV-1 proteins, intact virions in VCCs, RNA and R5-tropic integrated DNA, although viral component could not be detected in T-cells. Moreover, in the sQVAO, infectious HIV-1 production could be reactivated from urethral cells following stimulation with the macrophage-specific activator LPS, but not the T-cell-specific activator PHA. HIV-1 reservoirs form preferentially in a newly identified subset of polarized urethral macrophages we term transitional M1/M2. Such cells highly express IL-1-receptor, CD206 and IL-4-receptor, but lack CD163, and are increased in urethral tissues from HIV-1-infected cART-suppressed individuals. Finally, although macrophages form close cellular contacts with T-cells in these tissues, CD3 never co-localizes with CD68, indicating that during cART, HIV-1 in urethral macrophages does not result from phagocytosis / internalization of T-cells.

Conclusions: Our results show for the first time that despite suppressive cART, HIV-1 persists in urethral macrophages, which can be re-activated to release infectious virus. Hence, macrophages form a major HIV-1 reservoirs in the genital mucosa. These findings are determinant for therapeutic strategies aimed at HIV-1 eradication.

OP 4.5

In vivo massive expansion of a T-cell clone carrying a defective HIV genome: implication for the measurement of the HIV reservoir

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Background: HIV persists as an integrated genome in memory CD4+ T cells during ART. Infected cells have the ability to survive and to proliferate, thereby ensuring the long-term stability of the viral reservoir. Here we report the unique case of an HIV-infected individual on suppressive ART with a massive expansion of an HIV infected T-cell clone.

Methods: The participant (57 year-old African American man) received suppressive ART for more than 3 years at the time of leukapheresis.

Results: Measurement of HIV DNA in isolated CD4+ T cells by Alu-qPCR revealed an unusually high frequency of cells carrying integrated viral genomes (31,070 copies per million cells). In contrast, the frequencies of CD4+ T cells harbouring inducible HIV RNA by TILDA and replication competent virus by mQVOA were in the normal ranges of virally suppressed individuals (45 and 0.87 cells per million respectively), suggesting that the vast majority of the proviruses detected by Alu-PCR was defective. Circulating CD4+ T cell subsets [Naïve, Central, Transitional, Effector memory and Terminally differentiated cells (N, CM, TM, EM and TD, respectively)] were sorted by flow cytometry. Strikingly, 44% of EM cells harboured integrated HIV DNA, representing 97% of all integrated viral genomes in the blood of this participant. Single-genome sequencing of the envelope gene revealed a unique sequence in the EM subset. This was consistent with the identification of the massive overrepresentation of a specific integration site in chromosome 14, gene RPS6KA5. The massive expansion of a defective provirus suggested proliferation of a particular clone of EM cells, which was confirmed by TCR sequencing, with 60% of EM cells sharing the same rearranged TCR composed of a Vß2.1 chain. Cell sorting of Vß2.1+ EM cells revealed that 96% of these cells carried integrated HIV genome, confirming the clonal expansion of this HIV-infected T-cell clone.

Conclusions: These results indicate that massive clonal expansion of a single defective genome can occur in vivo, leading to a >35,000 fold difference between PCR and QVOA measurements. These proliferation events may contribute to the weak association between DNA measurements and replication competence in some individuals.

OP 4.6

Gut and blood differ in mechanisms governing HIV transcription/latency

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Background: The gut is a critical site for HIV replication and persistence. Tissue-specific environments likely impact the size and activity of the reservoir. We hypothesized that the mechanisms and degrees of HIV transcriptional blocks underlying HIV latency differ between the gut and blood.

Methods: We investigated the mechanisms that inhibit HIV transcription in vivo using a validated panel of RT-ddPCR assays to quantify HIV transcripts suggestive of transcriptional interference (U3-U5; 'read-through'), initiation (TAR), elongation (R-U5-tRNA; 'long LTR'), distal transcription (nef), completion (U3-polyA; 'polyA'), and multiple splicing (tat-rev). Total RNA and DNA were extracted from matched FACS-sorted CD4+T cells from blood and rectum (n=7) and from matched PBMCs and rectal biopsies (n=9); all samples were obtained from individuals on effective ART. Levels of each transcript were quantified and expressed as absolute copies/106 cells (normalized to a reference gene), ratios of each transcript to total (TAR) and processive (long) transcripts, and average transcription levels per provirus (HIV RNA/DNA).

Results: Rectal biopsies showed low levels of read-through transcripts (median=23 copies/10^6 cells) and a gradient of total (679)>elongated (75)>nef (16)>polyA (11)>multiply-spliced HIV RNAs (<1) [$p<0.05$ for all comparisons], demonstrating blocks to HIV transcriptional elongation, completion and splicing. Levels of total (TAR) transcripts per CD4+T cell and per provirus were significantly lower in the rectum compared to blood (median 2.7 vs. 31.8, p=0.016; and 3.5 vs. 15.4, p=0.008; respectively), indicative of lower HIV transcription initiation in the rectum. The ratio of total to elongated transcripts in CD4+T cells was 6-fold lower in rectum than blood (p=0.016), suggesting less of a block to HIV transcriptional elongation in the rectum. There was also a trend toward a lower ratio of read-through/elongated transcripts in rectal CD4+T cells (p=0.078), suggesting less transcriptional interference.

Conclusions: The blood and gut differ in relative contributions of mechanisms governing HIV transcription/latency, with a greater block to HIV transcriptional initiation in the gut (not due to transcriptional interference) but less block to elongation. These mechanistic differences may reflect tissue-specific variation in host gene expression, viral sequences, and/or extracellular milieu and are important to consider in designing therapies that aim to eliminate latent cells in all tissue compartments.

PP 4.0

Intrinsic resistance of HIV-1 to SAMHD1 restriction permits efficient macrophage infection

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Background: SAMHD1 is a dNTP triphosphorylase that limits dNTP pools in non-mitotic cells, such as macrophages, where dNTPs are excessive to need. Macrophages are natural targets for lentivirus infection and since reverse transcription of viral cDNA is dependent on adequate dNTP pools, lentiviruses must inactivate SAMHD1 in order to infect these cells. HIV-2 and many SIVs have evolved the accessory protein Vpx that targets SAMHD1 for proteasomal destruction.Vpr is a Vpx homolog expressed by HIV-1 yet HIV-1 Vpr does not appear to restrict SAMHD1.It, therefore, remains unclear how HIV-1 infects macrophages in the face of SAMHD1 restriction.

Methods: Methods used to test our hypothesis include flow cytometry, siRNA knock-down, dNTP measurement, lentiviral cloning, and qPCR.

Results: Here, we demonstrate that HIV-1 efficiently infects macrophages without modulating SAMHD1 activity or cellular dNTP levels. Furthermore, superinfection experiments indicate that macrophages infected by HIV-1 remain non-permissive to Vpx-deleted SIV.To test the hypothesis that the Km of HIV-1 Reverse Transcriptase (RT) allows efficient usage of cellular dNTPS, we created chimeric SIV and HIV-1 variants in which RT was exchanged. Vpx-deleted SIV containing an HIV-1 RT was restricted, suggesting that HIV-1 RT does not provide SAMHD1 resistance. However, SIV RT, within the context of HIV-1, was able to reverse transcribe in the presence of active SAMHD1.

Conclusions: This indicates that the HIV-1 genome harbors a determinant distinct from RT that confers intrinsic resistance to SAMHD1 thereby permitting efficient macrophage infection even in a low dNTP environment.

PP 4.1

Measurement and characterization of the latent reservoir for HIV-1 in patients receiving solid organ transplant

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Background: The latent reservoir (LR) for HIV-1 in resting CD4+ T cells is difficult to measure because current assays measuring HIV-1 DNA cannot distinguish defective viruses, and viral outgrowth assays which provide minimum estimates of replication-competent virus require large blood volumes. Newer limiting dilution PCR-based assays

use smaller samples and measure transcription-competent proviruses, but recent studies have shown that these still include defective viruses. To further limit measurement of defective viruses, we developed the quantitative viral induction assay (QVIA). We applied this assay to longitudinal samples from HIV-infected individuals receiving kidney or liver transplantation from HIV+ or HIV- donors while on complete viral suppressive therapy.

Methods: 4×106 resting or bulk CD4+ T cells were plated at limiting dilution and treated for 18 hours with PMA/ionomycin or vehicle alone. Mrna isolation, cdna synthesis, and qpcr for HIV-1 polyadenylated mrna were performed. QVIA uses a cycle threshold cutoff to increase specificity for intact, induced viral RNA. The number of inducible proviruses per million cells (IPPM) was estimated by qpcr positive wells at each dilution. Induced RNA from positive wells was sequenced by NGS in pol and gp41 regions to determine the most prominent viral strains in the well.

Results: Longitudinal patient samples were analyzed pre-transplant, and at 13, 26, and 52 weeks post-transplant (n=13). Immunosuppressive regimens were per standard of care, and for kidney recipients included antithymocyte globulin (ATG) for induction. Among 40 samples, median IPPM=6.301 (IQR=1.693-16.415). IPPM increased over time in 7 patients (ranging 2-10 fold) with 4 remaining elevated higher than baseline for 26 weeks or more. RNA positive wells from longitudinal samples (n=4) were sequenced with a median of 25 prominent strains per timepoint.Viral clones were maintained in the LR for all four patients, and in 3 patients there was an emergence of previously unseen viral variants post-transplant.

Conclusions: We demonstrate that QVIA can be used to measure the HIV-1 LR and characterize changes over time by sequencing RNA positive wells. Using QVIA we measured an increase in size of the LR in several patients post-transplant, an unexpected result in a cohort of patients receiving lymphocyte-depleting ATG treatment.

PP 4.2

Enrichment of HIV proviral DNA from mononuclear leukocytes for next-generation sequencing of integration sites

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Background: The reservoir of HIV infected cells in antiretroviral treated individuals represents a challenging hurdle for cure of infection. In infected individuals, clonally expanded, HIV-infected cells persist and comprise a significant fraction of the reservoir. Defining HIV integration sites (IS) could provide insight into genes that may be modulated by the provirus, which may contribute to the persistence of these clones. Sequencing of HIV IS can be challenging and costly due to the relative rarity of infected cells in treated individuals, especially in those who initiate ART during primary infection. We hypothesized that pre-sequencing preparation of DNA with the use of a 'bait' and labeling with 'primer-ID' will enrich HIV integration sites in DNA specimens and allow quantification of HIV IS and clonal IS.

Methods: An 180nt biotinylated DNA probe complementary to the HIV-1 LTR, Illumina adapter oligonucleotides with a 12 bp random primer-ID, and primers complementary to Illumina adapters and the U5 region of LTR were synthesized. Probes were hybridized to fragmented genomic DNA, and then combined with streptavidin-coated beads (Dynabeads™ M-270) for magnetic separation from DNA without HIV LTR. The HIV 3'-LTR-IS-human genome junctions were displaced from the probe by a single round linear extension. T-tailed Illumina sequencing adaptors were ligated to the human DNA using T4 DNA Ligase, followed by Illumina sequencing.

Results: In a contrived specimen (ACH2 and salmon sperm), one capture round with the LTR-bait concentrated HIV IS >10 fold by the removal of 96.6% of DNA. Similar treatment of human DNA with 85 copies of HIV LTR/µg removed 1.78 µg (60.5%) of DNA. Sequencing yielded 55 IS of which 37 were unique, and by virtue of unique Primer ID, 10 clonal IS.

Conclusions: Enrichment of the HIV 3'-LTR-IS-human genome junctions prior to sequencing, as show here, increases the efficiency of generating Illumina sequences of HIV IS.

PP 4.3

Infection of astrocytes by a virus isolated from CSF cells of an HIV-positive patient virologically suppressed with ART

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Background: Studies show that the brain is an important reservoir of HIV and CSF viral escape is broadly investigated as a predictor of the brain reservoir among HIV+ patients experienced with ART. However, HIV infection of astrocytes in vitro is inefficient. We investigated if HIV strains from the central nervous system were able to infect astrocytes.

Methods: 100-150 ml of CSF was collected via lumbar drain and the cells were cocultured with donor PBMCs for HIV isolation. An HIV isolate was characterized in cell lines and primary astrocytes. Sequencing was done for the HIV isolate and the PCR product from CSF cells in a follow-up.

Results: We collected CSF cells via lumbar drain from 7 patients with plasma viral suppression; 5 had normal cognitive performance and two had HIV-associated neurocognitive disorder (HAND). HIV was not detected in the CSF of any of these patients by clinical lab. We isolated an HIV from one patient with normal cognition after 10 days-coculture with PBMCs from heathy donors. This virus was able to infect MT2 cells and Jurkat-Tat (JKT) cells and induced syncytia, but could not infect THP-1-derived macrophages. These observations indicate that this isolate was an X4-tropic virus. Its X4 tropism was further confirmed by sequencing the env gene and prediction based on its V3 loop. When the virus stock was initially incubated with primary fetal astrocytes, no infection was detected. However, the virus could infect astrocytes via a transwell culture system where HIV-infected JKT cells were loaded on the top chamber and only HIV particles could go through its membrane and reach astrocytes in the bottom wells of plate. This is consistent with prior observations that immature HIV particles released from the infected lymphocytes were able to directly bind to CXCR4 on astrocytes in the absence of CD4 triggering virus-cell fusion and leading to the infection. Single genome amplification of HIV and sequencing was performed in CSF cells in a follow-up visit, but only defective genome (containing gag) was obtained.

Conclusions: Despite adequate antiretroviral therapy, low level HIV may be still present in the CSF that may predict viral reservoir in the brain.

PP 4.4

Replicate Aptima VL testing detects residual viremia in most ART-treated adults

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Background: The ability to determine if a curative intervention reduces the reservoir or if a latency reversing agent is effective will depend on access to ultrasensitive, high-throughput measurements

of residual viremia. Current viral quantification methods are limited by lack of sensitivity or the need for specialized, lengthy processing.

Methods: The Aptima HIV-1 Quant Assay provides standard viral load (VL) measurements on a 0.5 mL sample, but it also provides a reactive/non-reactive digital readout that may be reasonably sensitive even when only a single copy is present in the 0.5 mL sample. Readouts on multiple replicates (reps) on the system's automated platform using the standard sample volume can provide ultrasensitive estimates of copies/mL (cp/mL) via Poisson analysis. An analytical panel comprised of 5 serial dilutions each of 4 HIV+ samples was blindly tested using the Aptima Assay in 45 reps on 25 mL per dilution, and 110 large volume samples from antiretroviral-suppressed RAVEN study participants with consistently negative standard VL assay results were subjected to rep testing.

Results: Dilutions of the 4 samples calculated to range from 9 to 0.2 cp/mL had VL estimates generated via the 45-rep Poisson analysis that ranged from no underestimation to underestimation of the target concentrations up to 3-fold. On initial 9-rep testing of 110 samples from 59 ART-treated adults followed in the RAVEN cohort, 63 samples from 37 individuals had detectable VL. An additional 36 reps were performed on a subset of 19 samples, 7 of which were initially undetectable. Four of seven initially undetectable samples had positive results when tested with the additional reps. The Poisson-derived estimates in the 19 samples tested with 45 total reps ranged from 0 [95% CI 0 – 0.18] to 2.197 [95% CI 1.52 – 3.21] cp/mL.

Conclusions: The Aptima HIV-1 Quant Assay provides a high throughput means to quantify VL to <1 cp/mL with large volume plasma specimens which allows detection and quantitation of VL in most ART-suppressed patients. Given the assay's performance characteristics, its lack of reliance on specialized specimen handling and the highly automated approach, this assay is well-suited to early- and late-phase clinical trials of HIV curative interventions.

PP 4.5

Examining functional alterations of HIV-1 Tat variants associated with neurocognitively impaired patients in the Drexel Medicine CARES Cohort

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Background: While the mortality associated with HIV-1 infection has decreased, the incidence of neurocognitive impairment (NI) has increased. Previous studies have detected the HIV-1 protein Tat within the periphery, CSF, and brain of infected patients, even those adherent to antiretroviral therapy. Current studies seek to identify and characterize predominant genetic variations within Tat that correlate with NI and examine their functional impact on intracellular (promoter transactivation) and extracellular (secretion, neurotoxicity) functions of the protein.

Methods: HIV-1 Tat sequences were obtained from PBMCs isolated from patients with and without NI in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort. Given brain-derived Tat variants from demented patients have previously been shown to be unable to transactivate the HIV-1 LTR, five NI and five non-NI patients were selected based on their Global Deficit Score (GDS). Tat constructs were synthesized from their predominant PBMC-derived Tat sequences.

Results: Results have demonstrated no difference in LTR transactivation in these patient-derived Tats. Logistic regression models have predicted that specific amino residues correlated with the NI status of a given patient. These Tat variants are being produced and examined for alterations in transactivation of the viral LTR or host genes and impact on secretion and for extracellular activity in an animal model to assess alterations in behavior caused by injection of extracellular Tat within the prefrontal cortex (PFC). Results have demonstrated certain amino acid variants in Tat to reduce LTR transactivation.

Conclusions: Conclusion: Amino acids associated with Tats from NI patients have the potential to differentially alter selected protein functions and may be involved in determining whether individuals develop HAND.This work is supported by, NIMH P30 MH092177 (CNAC/CTRSC, Drexel Component PI, BW), NIMH T32 MH079785 (Drexel Component PI, BW), NIMH P30 MH092177 Developmental Grant (PI, SK) and R01 NS089435 (PI, MRN).

PP 4.6

Blinded evaluation of ultrasensitive assays of HIV in plasma

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Background: A major barrier to developing HIV cure interventions is the lack of validated assays that reliably quantify HIV in plasma below the limit of detection of current clinical assays.We sought to objectively assess the performance characteristics of newer, more sensitive assays to quantify plasma viremia.

Methods: The HIV Reservoir Assay Validation and Evaluation Network (RAVEN) project was created to assess the sensitivity, specificity, limits of detection and quantitative ranges of blood-based assays of HIV persistence.To accomplish this for HIV detection in plasma, blinded 50-sample panels containing subtype B and C HIV including duplicate, virus-spiked analytic standards (2 subtype-specific 5-step, 3-fold dilution series with highest concentrations ranging from 45 to 6 copies/mL to lowest concentrations ranging from 0.33 to 0.07 copies/mL), clinical samples with expected low-level viremia and negative controls were distributed to 8 laboratories for HIV quantification using 9 assays. Four assays included centrifugation to concentrate virus prior to HIV RNA nucleic acid extraction and PCR amplification; two included replicate testing using diagnostic HIV RNA assay platforms; while two utilized ultrasensitive detection of p24 Ag without virus enrichment. Results were analyzed for sensitivity, specificity, reproducibility, and ability to accurately quantify HIV in standards.

Results: Data from five laboratories using 6 assays were included in this analysis. Four of five RNA-amplification based assays detected virus in the standards down to ~1 copy/mL in at least 1 of the 2 replicates; negative controls were all negative. All RNA-amplification-based assays had strong correlations between replicates across the

standards (p<0.05, rho>0.8).Four assays quantified standards with little bias (mean recovery 69-218% of nominal HIV [RNA]) whereas one assay overestimated copies/mL by >300%. Ultrasensitive p24 Ag assays were not able to quantitatively measure HIV in the diluted standards. One p24 assay detected HIV protein in virus-spiked samples, with observed specificity of 90%.

Conclusions: p24 assays can detect virus in seronegative plasma without virus enrichment, but dynamic sensitivity was lacking at <45 copies/mL. Ultrasensitive RNA-amplification assays following virus enrichment or with replicate testing can quantitatively measure HIV RNA down to ~1 copy/mL, which is necessary to assess the impact of experimental curative interventions on residual viremia.

PP 4.7

HIV persistence in lymph nodes from virally suppressed individuals: residual production VS latency

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Background: HIV preferentially persists in follicular helper T cells (Tfh) from lymph nodes (LN) in virally suppressed individuals. However, whether these cells are latently infected or support low levels of viral production is still unclear. We evaluated the relative contributions of latency and viral production in LN from individuals on suppressive ART.

Methods: LN mononuclear cells were obtained by LN biopsies (n=6) or fine needle aspirates (n=4) from 10 virally suppressed participants on ART for 1.2 to 14.4 years. The frequency of Tfh cells was measured by flow cytometry in all samples. Relative contributions of residual transcription and latency were measured by Tat/Rev Induced Limiting Dilution Assay (TILDA) in total CD4 T cells using the unstimulated and stimulated versions of the assay, respectively. In addition, Tfh-enriched cells (CD45RA-PD-1+) and non-Tfh cells (CD45RA-PD-1-) were sorted by flow cytometry from the 6 biopsies. Frequencies of HIV-infected cells in sorted subsets were measured by alu-qPCR and TILDA.

Results: The frequency of Tfh cells tended to inversely correlate with the duration of viral suppression (p=0.07, r=-0.6). Within the total pool of CD4 infected T cells, the contribution of latently infected cells increased with the time spent on ART (p=0.007, r=0.81), whereas the contribution of cells undergoing residual production decreased (p=0,005, r=-0,81).Among individuals suppressed for >8 years from whom LN biopsies were obtained (n=6), Tfh cells were slightly enriched for HIV DNA compared to non-Tfh cells (median=3091 VS 1606 HIV DNA copies/million cells, respectively). Nonetheless, cells producing tat/rev RNA after stimulation were more rarely detected in the Tfh-enriched subset compared to non-Tfh cells (2/6 detectable measurements in Tfh VS 6/6 in non-Tfh). Overall, in individuals suppressed for >8 years, Tfh-enriched cells showed a modest contribution (12%) to the pool of cells containing inducible HIV genomes.

Conclusions: Our results indicate that residual levels of HIV transcription decrease with the time spent on suppressive ART and that the contribution of Tfh cells to the HIV reservoir may diminish after several years of viral suppression.

PP 4.8

HIV-1 populations can persist as active reservoirs in the CNS during ART treatment

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Background: Some subjects on antiretroviral therapy have detectable levels of HIV-1 in their cerebrospinal fluid (CSF) despite having undetectable levels in their plasma (i.e. CSF escape). Persistent CNS reservoirs may be a source of CSF escape virus.

Method: Illumina MiSeq deep sequencing with Primer ID was used to thoroughly and accurately characterize the env diversity and drug resistance of HIV-1 found in the CSF of two subjects on ART with CSF viral loads (VLs) >40 cp/ml and plasma VLs <40 cp/ml. In addition, single genome amplification (SGA) was used to generate full-length env clones from these viral populations and their macrophage tropism was determined based on their ability to enter Affinofile cells expressing a low density of CD4, similar to the level expressed on macrophage.

Results: One participant had detectable HIV-1 in their CSF when analyzed 22 and 30 months after ART initiation, despite having an undetectable plasma VL at both time points. Deep sequencing analyses revealed that the CSF escape virus from the two time-points formed a lineage that was diverse, genetically distinct from virus found in the plasma pre-therapy, and partially drug-resistant. Genetic analyses of viral populations in plasma at three time-points after ART initiation, but prior to plasma viral suppression, revealed emergence of a viral lineage that was genetically similar to the CSF escape virus population. Entry assays revealed that the CSF escape virus had an enhanced ability to utilize a low level of CD4 for entry. In contrast, the second participant had transient CSF escape observed at a single time-point 22 months after ART initiation and this CSF escape population was homogeneous, drug-sensitive and adapted to entering CD4+ T cells with a high density of CD4.

Conclusions: Our results indicate that CSF escape is a variable condition with different origins. In one participant, a CSF escape virus population was persistent during ART treatment and was likely produced by HIV-infected macrophage or microglia in the CNS. While the prevalence of persistent CNS reservoirs is unknown, our results indicate that such populations can evolve and persist in the CNS during ART.

PP 4.9

Genotypic and phenotypic characterization of replication-competent HIV clones from patients' reservoir

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Background: Current antiretroviral therapy allows the sustained control of viremia in most HIV-infected patient. Treatment interruptions almost invariably result in a rapid rebound of viremia to pre-therapy levels. Viral rebound is considered to originate from replication-competent proviral genomes integrated in resting memory CD4+ T-lymphocytes. Here we characterized clonal viruses from the reservoir of two patients.

Methods: Resting CD4+ T-lymphocytes were isolated from peripheral blood, stimulated, and cultured under limiting dilution conditions with donor CD4+ T-lymphocytes to allow virus outgrowth. Viral emergence was monitored by p24 quantification in the culture supernatant. Individual clonal viruses were isolated by short term culture, and their replication kinetics and per-particle infectivities were determined. The near full-length genome of individual clones was sequenced.

Results: Resting CD4+ T-lymphocytes from two HIV-infected and successfully treated patients were studied. Clonal replication-competent viruses from 12 wells from each patient were investigated. Within each patient, clonal viruses displayed different replication kinetics generating peaks between day 4 and 9 (patient 1), and between day 9 and 14 (patient 2). Also, for viruses reaching the peak on the same day, the amount of virus produced in the supernatant varied extensively. Single-cycle per-particle infectivities differed by 4-fold (patient 1) and 2-fold (patient 2). Near full-length genome comparison will be used to identify genetic determinants of the observed variances, taking advantage of the close phylogenetic relatedness of individual clones from each patient.

Conclusions: This work highlights differences in the genotypic and phenotypic properties of reservoir viruses from treated patients. The characterization of replication competent viruses is crucial for the design of strategies aiming the reduction of the HIV reservoir or the prevention of virus rebound.

PP 4.10

HIV integrates in genes regulating cell cycle, DNA damage, and viral transport

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Background: HIV integration is a key step in the viral replication cycle. Prior studies disagree on whether HIV preferentially integrates in oncogenes leading to clonal proliferation. We performed HIV integration site analysis from HIV+ participants and a humanized mouse model.

Methods: HIV+ adults on effective antiretroviral therapy (ART) were sampled from the UCSF SCOPE cohort. We extracted human DNA from PBMCs, enriched for CD4+ T cells, and quantified cell-associated HIV total DNA and unspliced RNA by qPCR. HIV integration sites were identified using nested PCR (Illumina MiSeq) with primers for HIV genome and linker sequences. A novel humanized mouse model challenged with a CCR5-tropic virus allowed in vivo comparisons of HIV integration. Sequences were aligned using UCSC Genome BLAT (hg38 assembly). Gene set enrichment analyses were performed using COSMIC Cancer Gene and Gene Ontology Consortium databases.

Results: Twenty-six (96% male) participants were included with median age of 46 years, timing of ART from HIV infection of 2.6 years, and ART suppression of 5 years. Among a total of 31,890 detected integration sites, there was a high degree of similarity in integration site loci (7,504 sites) and frequency between clinical and humanized mouse samples. Only 4.5% of integrations occurred within oncogenes. Integrations occurred in genes involved in cell cycle, DNA damage, membrane and nuclear envelope disassembly, viral transport, and DNA unwinding (all >2.26-fold enrichment, false discovery-adjusted P<1.21×10–2). The frequency of unique integration sites was inversely correlated with log10copies of unspliced HIV RNA (Spearman R=–0.52, P=0.0075) but not with total HIV DNA (R=–0.27, P=0.18). Timing of ART was also inversely associated with the frequency of unique sites (N=22, R=–0.44, P=0.039) but not with the %expanded clones.

Conclusions: In a large HIV integration site analysis, we observed consistent integration patterns between HIV+ patient and *in vivo* humanized mouse samples. The majority of insertions occurred in genes that may serve to promote viral latency, supported by the observation that a higher number of unique integration sites correlated with lower levels of residual viral transcription, and the observation that integrations were enriched in genes associated with cell cycle, response to DNA damage, and viral transport.

PP 4.11

HIV viremia is the product of a small fraction of infected cells

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Background: HIV persistence during ART is a substantial obstacle to HIV cure. Understanding dynamics of HIV viremia and infected T cells is essential for characterizing HIV reservoirs. Upon initiating ART, plasma viremia undergoes multiphasic decay kinetics reflecting loss of infected cells. HIV infected cells also decline but kinetics of decay during first weeks of ART have not been well characterized. In particular, it is not known what proportion of infected cells contribute to viremia. To address this issue, we developed sensitive and accurate multiplexed droplet digital approaches (ddPCR) to quantify early HIV-1 decay kinetics.

Methods: HIV infected ART-naïve individuals (N=10) enrolled in a clinical trial of 4-drug ART (2 NRTI+NNRTI+PI) at the NIH were frequently sampled prior to and upon ART intiation. HIV DNA from PBMCs obtained pre-ART, during first and second phase viral decay, and after viral suppression was quantified using ddPCR assays targeting HIV gag, LTR, and tat/rev. Plasma HIV RNA was measured (bDNA) concurrently. We analyzed the decay kinetics of HIV viremia and of cell-associated HIV DNA during first and second phase decay.

Results: All patients had successful suppression of HIV RNA to <50 cps/mL plasma by a median of 139.5 days on ART. Overall HIV DNA/million CD4+ cells decreased an average of 4.5-fold from pre-therapy to viral suppression. Strikingly, while HIV RNA in the blood declined on average 96% after 6 days on therapy, HIV DNA declined an average of only 30% (mean 491 cps HIV DNA/ml; range 49-903 cps/ml) indicating that the majority of viremia is produced by a small fraction of HIV infected cells, and that each infected cell is responsible for a median of 104 cps HIV RNA in plasma (range 21.2-8999 cps/cell). During second phase decay, virus production declined to c. 2.7 cps/cell (range 0.04-13.4 cps/cell).

Conclusions: Prior to ART, plasma viremia is the product of only a small fraction of HIV-infected cells. After initiating ART, HIV reservoirs responsible for persistent viremia are relatively limited and exhibit substantial variation in virus production. Analysis of early HIV RNA and DNA decay kinetics will be useful in characterizing patient-specific differences in establishing HIV reservoirs.

PP 4.12

Genetic diversity and CTL escape burden in the replication-competent HIV reservoir in youth in a therapeutic HIV vaccine trial

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Background: Genetic diversity and immune escape within the replication-competent HIV reservoir are barriers to cure; our knowledge of these parameters remains incomplete, particularly among infected youth. We longitudinally quantify sequence diversity and inferred CTL escape mutation burden in HIV clones retrieved from the resting CD4+ T-cell latent reservoir of youth enrolled in a historic phase 1 therapeutic HIV vaccine trial (PACTG/IMPAACT-P1059:MVA-HIV prime/Fowlpox-HIV boost). We additionally investigate possible vaccine-induced selective pressures altering reservoir genetic composition.

Methods: 208 partial Pol and 189 Nef sequences from latent reservoir clones pre- and post-vaccines in 4 perinatal- and 10 nonperinatally-infected youth were analyzed. The latent reservoir was sampled at up to 9 timepoints over up to 72 weeks while participants maintained pVL <50 c/ml on ART. Possible vaccine-induced perturbations in the reservoir, manifesting as ongoing HIV evolution and/or vaccine-induced loss/expansion of proviral subset(s) were investigated by testing within-host HIV phylogenies for molecular clock signal and compartmentalization pre-/post-vaccine. Inferred CD8+ T-lymphocyte [CTL] escape mutations were defined according to published reference lists of HLA-associated polymorphisms.

Results: Within-host Pol diversity was higher in perinatally (n=4) vs non-perinatally-infected (N=10) adolescents (nonsynonymous substitutions at average 9% vs. 2.5% codons respectively, p=0.0006). Nef diversity was comparable between infection modes (mean 7% overall). Inferred CTL escape mutation burden varied within and between hosts. Perinatal infections trended towards higher escape mutation burden in Pol (median ~50% vs. ~35% for nonperinatal); Nef escape burden was comparable between groups (median ~50% overall). All but one participant exhibited at least one example of susceptible and escaped forms of the same CTL epitope co-existing within their reservoir. Three participants exhibited possible evidence

of within-host evolution and/or vaccine-induced selection of certain HIV lineages.

Conclusions: Results suggest that HIV vaccines may have perturbed the reservoir in a subset of participants. The higher burden of Pol escape in perinatal infection suggests that reservoir escape burden in conserved, rather than in the variable, HIV regions may correlate with untreated infection time as the former regions escape more slowly. Targeting reactivated latent reservoir cells with HIV vaccines will require consideration for the complexity of within- and between-host reservoir diversity and escape burden.

PP 4.13

The role of APOBEC 3G/3F in shaping early HIV-1 reservoir landscapes

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Background: Little is known about the action and potency of APOBEC proteins at the earliest stages of HIV-1 infection.Here, we interrogated proviral HIV full-genome sequences for evidence of G>A hypermutations in women identified with hyperacute infections in Durban, South Africa through twice-weekly screening of high-risk individuals.

Methods: PBMC were available from two patients detected at Fiebig II: (1) peak viremia 2.6×106 HIV-1 RNA copies/mL, treated same day, (2) peak 7.7×105, remained untreated, and two patients detected at Fiebig V: (3) peak 150, on-treatment for 7 days, (4) peak 5.7×107, remained untreated. Longitudinal samples were available at 1, 6, and 12-month post-detection. Total PBMC DNA was extracted, diluted for single-template full-HIV-genome PCR, and Illumina deep-sequenced. 'Intact' proviral genomes were defined as the absence of deleterious mutations (out-of-frame indels, premature stop codons, large deletions). Hypermutated HIV genomes were defined by Los Alamos Hypermut 2.0.

Results: 287 proviral genome sequences were obtained; 41 had hypermutations (14%).Interestingly, APOBEC activity was not detected immediately post-infection:In two patients identified at Fiebig II, no hypermutated HIV genome was observed (study baseline, 2 and 7 days post-last HIV negative tests); in fact, at that stage, 10/12 proviral DNA detected were genome-intact (83%).In contrast, at Fiebig V, hypermutations were detected in 8-14% of the total HIV DNA pool.Treatment decreased the effect of APOBEC activity on HIV reservoirs, presumably due to the lack of induction of innate/type-1 IFN response and/or new infectious cycles. In both treated patients, frequency of hypermutated genomes decrease over time (14%-11%-0% and 0%-0%-0%) but increased in untreated patients (0%-47%-40%-50% and 8%-9%-0%-24%).In terms of individual G>A mutations sites, the entire length of the HIV genome demonstrated susceptibility to hypermutations; frequency distribution across genome was bimodal, where the vif-vpu region was less frequently mutated.APOBEC3G was also a stronger contributor to the overall G>A mutation events than 3F (median 89% vs. 11% per genome respectively).

Conclusions: APOBEC activity was not observed at the earliest stage of infection, but can subsequently affect as much as 50% of all HIV genomes in untreated patients. APOBEC3G had a stronger activity over -3F throughout the infection process.

PP 4.14

The latent reservoir as a genetically diverse archive recapitulating within-host HIV evolutionary history

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Background: Given the continuous nature of within-host HIV evolution and reservoir establishment, and the long-lived nature of latently-infected cells, the reservoir should comprise a genetically heterogeneous archive of within-host HIV evolution. This heterogeneity could complicate immune-based cure strategies but our understanding in this area remains limited, in part because methods to infer latent HIV sequence ages are lacking. We developed a phylogenetic method to reconstruct HIV integration dates and applied it to date reservoir sequences in persons with long-term viremia suppression.

Methods: The method involves inference and optimal rooting of a maximum-likelihood phylogeny from longitudinal within-host plasma HIV-RNA and reservoir sequences, followed by calibration of a linear model relating root-to-tip distances of the former to their sampling dates. The model is then used to convert reservoir root-to-tip distances to their establishment (integration) dates. Following rigorous validation we used the model to infer reservoir integration dates in two individuals with long-term viremia suppression sampled in-depth using single-genome-amplification over a ~20 year period. Reservoir sequences included HIV isolated from PBMC after >10 years on cART and from low-level viremia blips on cART (presumably representing HIV released from the reservoir).

Results: Putative reservoir sequences were interspersed throughout both within-host phylogenies and exhibited comparable diversity to pre-cart plasma RNA sequences sampled over 10 years. Historic within-host genetic bottlenecks were also recorded in the reservoir. Inferred proviral integration dates were consistent with the reservoir harboring both ancestral and recent lineages, with the oldest dating to 20 years prior to sampling. Sensitivity analyses confirmed that the method can be applied to as few as two plasma HIV-RNA timepoints and that it is robust to rooting approach, thus broadening its applicability.

Conclusions: Our method for reservoir dating provides a novel and powerful addition to the HIV persistence research toolkit and reveals a genetically diverse.

PP 4.15

Effector memory T cells contribute to monotypic residual plasma virus production during long-term suppression

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Background: Although antiretroviral therapy (ART) can limit HIV replication to below the limit of detection of clinical viral load assays, individuals experience residual viremia. Residual virus is comprised predominately of monotypic/identical viruses, but the cellular origin of this residual plasma virus and whether specific viral variants persist over time on ART is unknown. We aimed to: characterize the evolutionary dynamics of residual viremia over time in chronically infected, long-term suppressed individuals and decipher the cellular origin of this virus by evaluating proviral integration sites (IS) in immune cell subsets. We hypothesized that infected proliferating cells that persist during prolonged ART will consist primarily of effector memory T (Tem) cells and that these cells contribute to the monotypic residual viremia.

Methods: Plasma viral RNA from 8 time points of one chronically-infected individual with viral loads <50 copies/ml plasma was subjected to single genome amplification of the C2V5 env region. Sequences were assembled into a maximum-likelihood phylogenetic tree. Leukapheresis specimens collected after 6 yrs of ART were cell-sorted into naïve, central memory, and Tem cells. HIV IS were identified from sorted cells using multiple displacement amplification-integration site looping assay. The proviral C2V5 env sequences adjacent to IS were used to compare to plasma C2V5 sequences to identify the potential cellular origin(s) of plasma virus.

Results: Preliminary findings reveal residual plasma viremia was dominated by a large cluster of monotypic, CXCR4-tropic virions that persisted for at least 3 yrs of ART. Upon IS analysis, a link was found between this monotypic cluster and a provirus found only in Tem

integrated in MLLT3. The MLLT3 protein is responsible for transcriptional elongation in the absence of HIV Tat.HIV integration and dysregulation of MLLT3 may enable low-level production of residual viremia during ART.

Conclusions: Although from a single case, we found peripheral Tem may contribute to persistent monotypic plasma virus during ART. Ongoing studies will examine the genome integrity of this provirus. These findings reveal the potential contribution of peripheral Tem in contributing to HIV persistence during successful ART. Further analysis of integration sites in immune cells may elucidate the potential biological pathways manipulated by HIV to allow persistence during ART.

PP 4.16

Differences in the proviral HIV DNA between HIV monoinfected and HIV/HCV coinfected individuals

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Background: In HIV/HCV-coinfected patients, chronic HCV infection leads to increased T-lymphocyte immune activation compared to HIV-monoinfected patients, which may contribute to a higher susceptibility to the viral infection and increase the HIV viral reservoir. Our objective was to evaluate the influence of HCV coinfection in the HIV viral reservoir size in resting CD4+T-cells.

Methods: Cross-sectional study: 36 patients with HIV and HCV-chronic infection in the absence of treatment anti-VHC (group VIH+/VHC+) and 29 HIV-monoinfected patients that never had been in contact with HCV (group VIH+). Peripherical blood mononuclear cells were extracted and resting CD4+T-cells were isolated (CD4+CD25-HLADR-CD69-). Total DNA was extracted and the HIV viral reservoir size was measured (Alu-LTR PCR). Differences between methods were calculated with a generalized linear model.

Results: Patients were caucasic, mean age of 49 years, undetectable viral load(≥1 year), CD4+T-cells≥500 cells/mm3,≥6 months with the same treatment and 63,1% (n=41) were men. Median CD4+ and CD8+T-cells were 766 cells/mm3 (637-1040) and 845 (700-998) cells/mm3, respectively, ratio CD4/CD8=0,9 (0,8-1,1) and nadir CD4+T-cells 295 cells/mm3 (262-376). 38,5% (n=25) had treatment based on integrase inhibitors (INI) and 9,2% (n=6) treatment based in non-analogs. 69,2% (n=27) of HIV/HCV patients showed fibrosis F1 and the genotype 1 was the most prevalent (55,6%, n=20). 58,3% (n=21) of HIV/HCV-patients were infected through parenteral route differing of the HIV group that never had been infected through parenteral route and 86,2% (n=25) of them were infected by sexual route (p=0,015). A higher infection time was observed in HIV/HCV than HIV-infected patients [171 months (45,7-346,1) vs.141,7 (82,9-247,9), respectively; p=0,026]. HIV/HCV patients presented an increased in the number of copies of proviral HIV-DNA compared with the HIV+ control group (203,4±47,1 vs. 87,35±22,53, respectively; p=0,026) confirmed by the multivariant analysis that also showed an association between the number of copies of integrated HIV DNA and the time of infection (p=0,016).

Conclusions: HIV/HCV patients showed a higher size in the proviral HIV DNA than HIV-monoinfected individuals that could lead to greater complexity in the elimination of the HIV reservoir in HIV/HCV-coinfected individuals

PP 4.17

Temporary ART initiated during primary HIV-1 infection limits the viral reservoir but increases virus diversity upon therapy interruption

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Background: Early initiation of ART is one of the most promising strategies for an HIV cure. Temporary ART initiated during primary HIV-1 infection (PHI) lowers the virological set point and defers the restart of ART during chronic infection (CHI). To elucidate the mechanisms behind these effects, we measured the virus diversity and reservoir size in patients treated with temporary ART during PHI.

Methods: Levels and HIV genetic diversity of plasma viral RNA, cell-associated (CA) HIV RNA and DNA were analyzed in HIV-infected patients who had participated in a randomized controlled trial of 24 or 60 weeks of temporary ART versus no treatment during PHI and subsequently (re)started ART during CHI after a median of 2,5 years without treatment. We performed single-genome sequencing of HIV-1 p6-PRO-RT region (1,42 kb) and estimated nucleotide and amino acid diversities by computing mean pairwise distances.

Results: First, we compared the on-ART proviral diversities in the same patients and at the same time points on ART between PHI and CHI ART periods. CA HIV DNA diversity was significantly lower during PHI ART than during CHI ART (p=0,023, Wilcoxon signed rank test). At the virological set point (36 weeks after ART interruption or randomization), levels of plasma HIV RNA, CA HIV RNA, and CA HIV DNA were all significantly lower in patients who had been treated with temporary ART as compared to the untreated patients. No significant difference was observed in the HIV nucleotide diversity of these markers between the treated and untreated patients. Surprisingly, the amino acid diversities of plasma HIV RNA (PRO) and CA DNA (PRO-RT) were significantly higher in treated vs. untreated patients (p=0,005 and p=0,028, respectively, Mann-Whitney test). These effects were not mediated by drug-resistance mutations as none were found in PRO or RT in any patient after early ART interruption.

Conclusions: Temporary early ART resulted in lower HIV reservoir but higher virus diversity after treatment interruption compared to the patients who did not receive early ART. Early ART might have augmented HIV-specific cellular immune responses, resulting in the development of immune escape HIV variants with reduced viral fitness upon ART interruption.

PP 4.18

Genetically intact but functionally impaired HIV-1 Env glycoproteins in the T-cell reservoir

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Background: The HIV T-cell reservoir harbors only a small percentage of intact HIV genomes, but little is known about the functional capacity of viral proteins expressed by these sequences.We have focused here on expression and function of the HIV envelope glycoprotein, a major target for the host immune response and a strong effector of HIV cytopathicity.

Methods: Resting CD4+ T-cells were collected from 4 HIV1-infected subjects in whom viral replication had been fully suppressed by antiretroviral treatment for more than 4 years. After in vitro T-cell

stimulation, Env sequences were obtained from two sources: clonal replicative virus isolated by qVOA, and cell-associated mRNAs isolated by limiting-dilution PCR. Env sequences were cloned in a Rev-independent expression vector.Env expression was examined quantitatively by flow cytometry, Western Blot and immunofluorescence. Env function was tested using a quantitative cell-cell fusion assay and a pseudotype infectivity assay.

Results: All qVOA-derived Envs were intact, while 26% of mRNA-derived Envs carried lethal, mostly A-to-G, mutations. Only clones with intact sequences were tested. As expected, clusters of identical sequences were seen in all 4 patients and Env diversity was widest in patients with longest HIV history. Env fusogenicity was highest in qVOA viruses, with 90% of them expressing fusion values above 50% compared to NLAD8 control. Fusogenicity was significantly lower among mRNA-derived Envs: only 41% reached fusogenic levels above 50% of control, 27% of them had fusogenic levels below 25% of control, and 14% had no detectable fusion activity. Similar findings were observed in a pseudotype infectivity assay, with a significant correlation. Flow cytometry, immunofluorescence and Western-Blot analyses revealed that defects in Env function were essentially related to defects in Env expression, maturation and/or stability.

Conclusions: The discovery of apparently intact, yet partially or fully defective, Env sequences in the HIV reservoir has two implications. First, Env fusogenicity and cytopathicity could be one important factor driving selection of defective HIV genomes in the reservoir. Second, the presence of poorly expressed and poorly functional Env sequences in the reservoir could preclude immune elimination of cells harboring these sequences in « kick and kill » HIV eradication attempts.

PP 4.19

Pacific biosciences small molecule real-time (SMRT) deep sequencing detects significant viral population structure in brain *vs* non-brain autopsy tissues from combined antiretroviral therapy (cART)-positive subjects

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Background: The presence and location of sanctuary sites for HIV during cART are unclear. A sanctuary likely contains long-lived cells, has limited drug penetration, and is an evolutionary compartment prior to cART; all features consistent with the brain. We used PacBio deep sequencing to investigate full-length HIV env in 4 brain and 7 non-brain autopsy tissues from two cART+ subjects (s123 and s162).

Methods: Full length env from proviral DNA was PCR amplified for template in a SMRT sequencing reaction. Circular consensus reads were filtered at 99% quality, 10 passes, and >2000 bp. Reads were aligned using MAFFT and manually edited for a sequence total of 52,842 (s123) and 34,889 (s162). Sequences were clustered using HIV-TRACE at three different thresholds of genetic similarity (0.1%, 0.55%, & 1.0%).Maximum likelihood phylogenies were inferred using PhyML. Charge, length, and number of glycosylation sites were calculated for env variable regions.

Results: Brain-derived sequences showed significant population structure compared to non-brain. At the lowest clustering threshold (0.1%), parietal (P), occipital (O), and frontal (F) lobe sequences were in separate clusters for s123. At 0.5% and 1%, P/O clustered together exclusive of any non-brain sequences, while F continued to cluster alone. In s162, one cluster contained all P sequences, as well as 3% of lymph node (LN) sequences at 0.1%. At higher thresholds, additional LN and colon sequences also grouped with the P sequences. Different charge and glycosylation site patterns characterized brain and non-brain tissues.

Conclusions: SMRT sequencing enabled an extensive analysis of complete HIV env sequences after cART. Brain-derived virus is a distinct population with potential functional differences that may impact persistence.

PP 4.20

Association between time spent with residual viremia after achievement of virological suppression and type of first-line antiretroviral regimen

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Background: To investigate if starting ART with different regimens, differently affects the time spent with residual viremia (RV) after achieving <50 HIV-RNA copies/mL.

Methods: Retrospective cohort study on prospectively followed HIV-infected patients who started ART since March 2009 with boosted protease inhibitor (PI/r)-, non-nucleoside reverse transcriptase inhibitor (NNRTI)-, integrase inhibitor (InSTI)-based triple regimens or with >3 drugs (>3 ds). Follow-up (FU) was censored at virological failure (VF=2 consecutive HIV-RNA≥50 copies/mL) or treatment discontinuation (TD), lost to FU, death or data freezing (March 2017). RV defined as any detectable HIV-RNA value below 50 copies/mL, as assessed by kPCR or Abbott Real-Time PCR.

The percentage of time with RV (%RV) was calculated as the cumulative FU time spent with RV on the observed FU. %RV were estimated and compared by use of the generalized linear model, after adjustment for pre-ART HIV-RNA, BL HIV-RNA (undetectable vs RV), rate of HIV-RNA decline since ART start, nadir CD4+ count, BL CD4+/CD8+, previous AIDS diagnosis, NRTI backbone, BL calendar year, age, gender, HCV-Ab.

Results: 771 patients (33%, 32%, 30%, 5% receiving PI/r-, NNRTI-, InSTI-based triple regimens or >3 ds, with a median (IQR) pre-ART HIV-RNA=4.66 (4.10-5.13) $\log_{10}$copies/mL, were included in the analysis.

After 1.57 (0.97-2.46) years of FU, adjusted means (95%CI) of %RV were 38% (30%-45%), 24% (16%-32%), 27% (20%-35%), 44% (33%-55%) in the PI/r, NNRTI, InSTI and >3 ds group (Figure1): %RV was significantly lower in patients treated with InSTIs (p=0.007, p=0.006) or NNRTIs (p<0.0001, p=0.0002) than with PIs/r or >3 ds.

VF and TD occurred in 7%, 3%, 3%, 8% (p=0.12) and in 80%, 34%, 18%, 92% of patients in the PI/r, NNRTI, InSTI and in the >3 ds group (p<0.0001).

Conclusions: First-line regimens based on PIs/r or on >3 ds are associated with a greater % of time spent with RV after achieving <50 HIV-RNA copies/mL.

PP 4.21

Spread of HIV-DNA in CD4+ T-cells subsets depends on ART initiation timing

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Background: Life-long resting CD4+ T-cells are the major long-lasting reservoir in HIV-infected individuals. We aimed at analyzing the dynamics of HIV-DNA in CD4+ T-cells subsets in individuals on successful dolutegravir-based regimen over 48 weeks.

Methods: Individuals in acute (AI) or chronic (CI) infection, and patients in virological success (VS) or failure (VF) on ART who initiated a dolutegravir-based regimen, were enrolled (NCT02557997). Cells

from baseline and week 48 of successful treatment were sorted in effector memory (TEM), transitional memory (TTM), central memory (TCM) and naïve (TN) CD4+ T-cells for total HIV-DNA measurements. Bayesian methods were used to estimate the probability (Pr) that HIV-DNA decreased for more than 0.25 log copies/10∧6 cells at week 48.

Results: Twenty-seven participants (8, 5, 10, 4 individuals in the AI, CI, VS and VF group, respectively) were included. At baseline, the highest contributions to the HIV-infected pool of CD4+ T-cells were observed in TTM cells in the AI group (62.8%), but in TCM cells for the CI, VS and VF groups (58.8%, 57.9%, 55.8%), respectively (Figure). After one year of dolutegravir-based regimen, TTM cells for the AI group (60.2%) and TCM cells for the CI, VS and VF groups (55.2%, 63.2% and 73.6%, respectively) still represented the main HIV reservoir. In these respective cells, HIV-DNA declined after 48 weeks of treatment in the AI group (4.00 to 3.26 log copies/10∧6 TTM cells, Pr>99%), in the CI group (4.07 to 3.57 log copies/10∧6 TCM cells, Pr=92%) and in the VF group (4.13 to 3.84 log copies/10∧6 TCM cells, Pr=59%) but not in the VS group (4.19 to 4.12 log copies/10∧6 TCM cells, Pr=8%).

Conclusions: HIV-DNA was mainly confined to TTM or TCM cells, when ART was introduced in acute or chronic HIV infection, respectively. HIV-DNA decreased after 48 weeks of dolutegravir-based regimen in all individuals except for switch cases.

PP 4.22

Disease-specific HIV Nef identified in multiple patients with neurological disorders and cancers

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Background: Although HIV infection promotes several macrophage (MO)-associated comorbidities, the viral mechanism driving these specific processes remains unknown. HIV-associated neurological disorders (HAND) are linked to M1-activated inflammatory MOs whereas HIV-associated cancers are linked to M2-activated anti-inflammatory MOs. The HIV Nef protein is maintains a variety of functional domains. We hypothesized that subtle changes within these domains stimulate MOs towards specific disease pathways. We used nonlinear models trained using machine learning to distinguish Nef sequences from patients with either HAND or cancer, based on slight physicochemical shifts within functional domains.

Methods: 2020 sequences derived from 31 subjects and 25 types of anatomical tissues were studied. For 23 subjects, the primary pathology, other than HIV infection, was known. Brain sequences were available from 10 subjects with HAND. 71 different physicochemical amino acids scales were analyzed for 10 functional domains of Nef. Evolved neural networks (ENNs) were applied to training and testing data sets to classify 1) brain from non-brain sequences and 2) cancer from non-cancer sequences. The best ENN was assayed for performance on the training, testing, and a third validation dataset of sequences with unknown etiology. Each sequence was scored and a binary threshold was used to discriminate samples into one of the two classes.

Results: Nef sequences from subjects with either HAND or cancers at death were classified with 84.5% and 90% true positive accuracy, respectively. Our approach elucidated structure-function properties of disease specific Nef that were not discernable from primary sequence information alone. Modifications in several functional domains were associated with brain-derived Nef; whereas the AP2 binding domain alone strongly associated with cancer-derived Nef.

Conclusions: Underlying physical and chemical differences of Nef are associated with specific pathologies, suggesting that a Nef-associated mechanism may promote MO-associated disease pathways. Disease-associated Nef will be useful as a biomarker and in biological assays to design novel drug targets to treat HIV-associated comorbidities

PP 4.23

HIV-DNA, CD32a CD4+ T-cells and immune activation on successfull dolutegravir-based regimen

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Background: The impact of antiretroviral therapy (ART) including novel integrase inhibitors on HIV reservoir is not clearly characterized. Here, we assessed the dynamics of HIV reservoir and immune activation markers among HIV-infected adults initiating a dolutegravir-based regimen (DBR) at different stage of HIV infection.

Methods: The DRONE study was a multicenter prospective study enrolling individuals starting a DBR and followed-up during 48 weeks (W48) (NCT02557997). Total HIV-DNA in peripheral blood mononuclear cells (PBMCs) and serum immune activation biomarkers (sCD14, sCD163, IL-6us and IP-10) were measured. CD32a+ CD4 T-cells were sorted in a subgroup of participants. Bayesian analysis was used to estimate the posterior probability distribution of HIV-DNA levels.

Results: A total of 169 participants achieved or maintained virological control on DBR. According to the clinical stage of HIV infection, participants were assigned to different groups: acute infections (AI, n=20), ART-naïve chronic infections (CI, n=21), individuals in virological success on ART (VS, n=116) and virological failures at baseline (VF, n=12). Using Bayesian mixed linear modeling, HIV-DNA decreased at W48 of DBR in the AI group (median decrease: -1.4 log copies/10∧6 PBMCs), the CI group (-0.5) and the VF group (-0.4), but not in the VS group (±0.0), with a probability of a clinically relevant decrease of at least 0.5 log of 100%, 93%, 47% and 0%, respectively (Figure). To further investigate the size of HIV reservoir in the VS group, CD32a+ CD4 T-cells were sorted in a subset of 10 individuals. Stable expression of high levels of CD32a were observed between baseline and W48 (median of 143/10∧6 of resting CD4 T-cells). Activation biomarkers decreased on DBR for individuals with ongoing HIV replication at baseline.

Conclusions: Successful DBR produced a rapid HIV-DNA decline in ART-naïve but also in ART-failing individuals. Though, dolutegravir failed at reducing HIV reservoir levels when introduced as a switching regimen.

Session 5: Immunology of HIV persistence

OP 5.1

Platelets from HIV-infected cART-treated patients carry infectious viruses and predict poor immunological recovery

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Background: Beside hemostasis, human platelets carry important immunological functions by interacting with immune cells. Furthermore, platelets interact with infectious pathogens and internalize HIV in vitro. Here, we investigated whether platelets from HIV-infected patients under Combined Antiretroviral Therapy (cART) carry infectious HIV and how it correlates with patient immunological status.

Methods: Infectious HIV content in platelets was assessed by PCR, Flow-FISH, confocal and electron microscopy, quantitative flow cytometry and infection-reporter cells using platelet-rich-plasma (PRP) from 75 cART-treated patients. Human primary macrophages were co-cultured with PRP without or with the platelet activation-blocker Abciximab to assess capacity of platelet-containing HIV to transfer and propagate infection. The presence of HIV in platelets was correlated with patient clinical status.

Results: Platelets from cART-treated patients with detectable but also undetectable viral loads enclose infectious viruses as detected consistently by viral RNA, p24 expression, and localization within platelet internal compartment. Functionally, platelet sheltering HIV can propagate productive infection to macrophages in-vitro. Blocking platelet activation with the therapeutical agent Abciximab prevents this transfer. The occurrence of HIV-containing platelets is independent platelet numbers whereas it strongly correlates with sustain low blood CD4+T-cell counts (<350 cells/microL in a 3 years survey) and nadir (<200 cells/ml). Accordingly, 65.2% of patients sheltering HIV in platelets do not respond immunologically to cARTand fail to restore a proper immune status over 18 months with a >20-fold higher likelihood than patients without HIV in platelets (OR:21.3, 95%CI:4.4-103.1, p<0.001).

Conclusions: This study not only sheds new light on the biology of HIV pathogenesis, describing an alternative pathway for viral dissemination in which platelets act as carriers of infectious viruses and as new players in cell-to-cell HIV transmission, but also suggests new diagnostic and therapeutic perspectives to improve immunological recovery in cART-treated patients.

OP 5.2

Follicular regulatory T cell dynamics in peripheral blood and lymphoid tissue during very early treatment initiation in HIV-1 clade C infection

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Background: HIV eradication efforts have been unsuccessful due to virus persistence in cellular and tissue reservoirs. Recent evidence suggests that germinal centres (GCs) within lymph nodes (LN) contain a novel subset of regulatory T cells (Tregs), termed follicular regulatory T (TFR) cells. These cells control the magnitude and specificity of the GC response and like Tregs are essential for the maintenance of self-tolerance and immune homeostasis. However, the immunosuppressive role of TFR cells in HIV infection and their contribution to viral control is not completely understood, particularly in clade C infection. Thus, we set out to investigate TFR cells using LN and peripheral blood (PB) samples and further determine the effect of early treatment on the frequency and function of this cell subset.

Methods: We analysed 16 individuals, grouped into HIV uninfected and HIV infected early and late ART initiators, from a clade C HIV infection cohort in Durban, South Africa. TFR phenotype, antigen specificity and localization were defined using flow cytometry, MHC Class II tetramers and immunohistochemistry (IHC) respectively. TFR regulatory cytokine; IL-10, was quantified using digital droplet PCR (ddPCR).

Results: Greater TFR cell frequencies were found in LN compared to PB samples ($p \leq 0.0001$) in all individuals. Very early ART initiators displayed larger proportions of TFR in the GC (PD-1^{hi}) compared to late treatment initiators, who had a PD-1^{lo}, follicular mantle phenotype($p \leq 0.04$). This result was confirmed by IHC. TFR cells produced more IL-10 and less IL-21 transcripts from ddPCR results consistent with a T regulatory functional profile. Increased IL-10 production by TFR was also observed in early treatment.

Conclusions: Our data shows that very early ART results in increased TFR cell numbers in the GC, where they likely influence the germinal centre response through interactions with follicular helper T cells (TFH). These results could have implications for immunotherapeutic interventions aimed at using TFR and TFH cells as potential targets.

OP 5.3

High-throughput single-cell transcriptome analysis of immune cells from HIV-1 infected individuals before and after therapy

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Background: The eradication of the latent virus reservoir is a key barrier to an HIV-1 cure, but the molecular signatures and pathways of latently infected cells not completely understood. Identification of biomarkers enriched in latently infected cells will allow for the monitoring and targeted of the latent reservoir for eradication. Here, we utilize single-cell RNA-seq to determine the transcriptomes of immune cells from HIV-infected individuals before and after ART to identify transcriptionally unique clusters of cells that may express candidate biomarkers.

Methods: Over 100,000 individual peripheral blood mononuclear cells (PBMCs) from chronically HIV-infected (6), HIV individuals on ART (3) and HIV seronegative individuals (3) were subjected to single-cell RNA-seq using the oil-emulsion encapsulation (10X Genomics) and high-throughput sequencing (Illumina).

Results: Single-cell transcriptome analysis demonstrated that immune cells from infected individuals are significantly altered during infection when compared to seronegative individuals. ART initiation restores some of the molecular pathways to that of seronegative individuals, but many immune cells remain altered during ART when compared to seronegative individuals. Moreover, we identified unique molecular biomarkers of actively infected cells as determined by HIV Gag mRNA expression compared with HIV negative individual cells.

Conclusions: We have established high-throughput RNA-seq at single-cell resolution. Using this technology we have identified molecular pathways altered during HIV-1 infection with and without ART. Single-cell resolution has also determined biomarkers of HIV-infected cells within the same donors. Future studies using this approach on cell populations enriched in the latent pool may yield novel biomarkers for the identification of the HIV latent reservoir.

OP 5.4

BCL-2 inhibitor sensitizes the latent HIV reservoir to elimination by CTLs

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Background: 'Kick-and-kill' strategies have shown promise in primary-cell models of latency, but have yet to show broad success in ex vivo patient samples. Our studies have found that latent reservoirs resist elimination, despite treatment with combinations of CTLs targeting non-escaped HIV epitopes and strong LRAs. Cummins et al recently observed elevated levels of the anti-apoptotic protein BCL-2 in CD4+ TCM-cells from HIV-infected individuals, which may increase infected cell resistance to elimination. Here, we investigate the ability of BCL-2 inhibitor 'venetoclax' to remove this barrier.

Methods: Reservoir reduction was assessed in an HIV eradication assay using various CTL immune effectors in combination with/without venetoclax and the following lras: bryostatin, PMA/Ionomycin, and CD3/CD28 antibodies. Resting CD4+ T-cells were co-cultured with lras and ctls for 3 days with arvs, and then measured for cell-associated HIV DNA (ddpcr) and replication competent virus by quantitative viral outgrowth assay (QVOA).

Results: Treatment of a primary cell model of latency model (Cultured TCM model), with either bryostatin alone or bryostatin with venetoclax, led to significant decreases in HIV DNA and replication competent reservoir. In ex vivo samples from ARV-treated participants, treatment with LRAs in combination with CTL immune effectors led to significant decreases in HIV DNA ($p<0.0001$, n=9), but no decreases in replication competent virus were observed (trend towards increase, $p<0.13$, n=9). Treatment with CTL clones, CD3/CD28 antibodies, and venetoclax together led to significant decreases in HIV DNA and in replication competent virus (HIV DNA: $p<0.001$, IUPM: $p=0.0009$, 4-fold decrease).

Conclusions: HIV eradication assays utilizing potent CTL immune effectors in combination with strong LRAs significantly depleted HIV DNA, but venetoclax was necessary (but not sufficient) to significantly deplete the replication competent reservoir from ex vivo CD4+ T-cells. These data indicate that some latent reservoirs may exhibit BCL-2-mediated resistance to elimination by CTLs. Further study is needed to explore venetoclax as an adjunct to kick-and-kill therapies.

OP 5.5

Defining the nature of protective CD8+ T-cell response in lymph nodes of HIV elite controllers

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Background: Cytolytic activity of peripheral blood CD8+ T cells has been closely linked to viral control in HIV elite controllers (EC). However, the vast majority of HIV replication in EC likely occurs in lymphoid tissue, where CD8+ T cell surveillance mechanisms are undefined. Here we assessed the functional and phenotypic properties of HIV-specific CD8+ T cells in the lymphoid tissues of elite controllers.

Methods: We obtained human peripheral blood mononuclear cells (PBMC) and lymph node mononuclear cells (LNMC) from HIV-infected EC, non-controllers and ART-treated individuals (ART). We performed multi-parametric flow cytometry and immunohistochemistry to define the phenotypic and functional profile of HIV-specific CD8+ T cells as identified by MHC-class I tetramers or responsiveness to peptide stimulation.The results were analyzed using FlowJo, GraphPad Prism, SPICE, and R Studio.

Results: We here demonstrate that memory CD8+ T cells in LN of EC do not express high levels of cytolytic proteins, such as perforin and granzyme B. These low levels of perforin and granzyme B are likely due to the low level of IL-15 present in LN of EC. In addition, these cells display poor killing capacity in a redirected cell lysis assay. While high frequencies of HIV-specific CD8+ T cells were detected in lymphoid tissue, these cells very rarely express perforin and granzyme B. Instead, they possess high levels of non-cytolytic polyfunctional responses directed against HIV peptides. Such responses were low or absent in non-controllers or those on ART. HLA-B27/57+ EC demonstrate a selective and high frequencies of CD8+ T cells targeting immunodominant Gag epitopes. Finally, HIV-specific LN CD8+ T cells from EC do not have enhanced ability to enter B cell follicles as defined by the expression of the chemokine receptor CXCR5, nor are those CXCR5+ cells more cytolytic compared to non-controllers and those on ART.

Conclusions: Together these findings redefine previous concepts of CD8+ T cell mediated control of HIV disease progression. During established infection, HIV appears to be controlled in tissues by non-cytolytic rather than cytolytic mechanisms. Knowledge regarding how HIV is controlled in this setting should be used to inform the identification and development of potentially curative interventions.

OP 5.6

Susceptibility to neutralization by bnAbs orrelates with infected cell binding for a panel of clade B HIV reactivated from latent reservoirs

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Background: Antiretroviral therapy (ART) suppresses HIV replication, blocking progression to AIDS. Efforts to cure infection are obstructed by reservoirs of latently infected CD4+ T cells that can re-establish viremia. HIV-specific broadly neutralizing antibodies (bnAbs), defined by unusually high neutralization breadths against globally diverse viruses, may contribute to the elimination of these reservoirs by binding to reactivated cells, targeting them for immune clearance. Few studies have assessed the reactivity of bnAbs against viruses reactivated from reservoirs. The relationship between neutralizing activity against reservoir isolates and binding to corresponding viruses infected primary CD4+ T cells is unknown.

Methods: Quantitative viral outgrowth assays (QVOAs) were performed with CD4+ T cells from participants on long-term ART from a clade B-infected cohort from Toronto. A panel of bnAbs were tested for surface binding to cells infected with 36 reservoir isolates by flow cytometry, and for neutralizing activity against the same viruses using a TZM-bl assay.

Results: PGT121 and 10-1074 potently neutralized 66-67% of reservoir isolates with geometric mean IC80s between 0.6-0.7 mg/ml. PG9 and PGDM1400 showed less coverage (39%-51%), with IC80s of 1.8 mg/ml and 6.3 mg/ml, respectively. CD4 binding sites antibodies displayed the broadest neutralization profiles - 63-97%, but with higher IC80 values of 4.6-16.7 mg/ml. The MPER antibodies showed poor neutralization, IC80s of 9.3-49.2 mg/ml.

We tested infected cell binding for all bnAbs at both 5 µg/ml and at their geometric mean IC80 neutralization concentrations. PG9, 10-1074, PGT121, 2G12, and PGDM1400 showed 58-72% coverage, with the former 3 bnAbs showing particularly strong binding. Broader binding capacities (72-81%) were observed for most CD4 binding sites antibodies, except for VRC01 (19%). The MPER bnAb 10E8 exhibited 66% coverage, but with background binding to uninfected cells. Combination of two bnAbs, e.g. CD4bs bnAbs with V3-Glycan bnAbs or V1/V2 bnAbs, resulted in coverage of up to 100% reservoir isolates.

With the exceptions of 2G12, CAP256, 2F5 and 4E10, all bnAbs exhibited strong correlations between binding capacity and neutralizing activity ($p<0.0001$).

Conclusions: We observed substantial heterogeneity in the binding and neutralization profiles of different bnAbs to reactivated reservoir viruses. Our results provide guidance on the selection of bnAbs for interventional cure studies.

PP 5.0

Retinoic acid (RA) upregulates α4β7 on CD4+ T cells and activates latent reservoirs

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Background: Current HIV cure studies are focused on the eradication of the viral reservoir but are limited by low sensitivity and poor reproducibility. This is partly due to the low frequency of cells harboring latent replication competent virus. Furthermore, the process of reactivation of the latent reservoir remains entirely stochastic resulting in an underestimation of the size of the viral reservoir.

Methods: We have developed an approach that seeks to improve the sensitivity based on upregulating α4β7 on CD4+ T cells.Here we utilized PBMCs from SIV infected rhesus macaques that were a) ART treated, b) those that spontaneously developed low viral loads, and for purposes of control PBMCs from SIV naïve macaques.

Results: The PBMCs were activated in vitro using anti-CD3/CD28+IL2 in the presence/absence of retinoic acid (RA). Viral loads were measured using RT-PCR assays, levels of p27 was quantified using ELISA assay and flow cytometry was utilized to measure α4β7 expression levels. The addition of RA led to>2-fold increase in the density of α4β7 expression by CD4+ T cells in all cultures. Of interest was the finding that the addition of RA to cultures of PBMCs from SIV-infected/ART-treated and the spontaneously low viral load macaques led to a 3-fold and 5-fold increase, respectively, in p27 levels in RA-treated activated cells compared to cultures without RA. Finally, the in vivo administration of RA to macaques with low viral loads led to a moderate increase in plasma viral loads.

Conclusions: We conclude that targeting the RA pathway can be a useful approach to improve the efficiency of currently used methods to upregulate α4β7 expression and activate latent reservoirs.

PP 5.1

HIV antibody and T cell responses on ART are associated with HIV DNA but not RNA

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Background: HIV-specific immune responses decline after initiation of ART. Infected cells that persist are thought to be invisible to the immune system, but if there is intermittent antigen expression and recognition, HIV antibody (Ab) and T cell responses may correlate with measures of HIV persistence.

Methods: Plasma and PBMC samples were obtained from 100 individuals on suppressive ART in the ACTG A5321 cohort. Cell-associated (CA) HIV DNA and unspliced RNA levels and Ab to Env/p24 were measured longitudinally at yr 1, 4 and 6-15 after ART initiation. Plasma HIV RNA by single copy assay and T cell responses (IFN-γ ELISPOT) against Gag, Pol, Env, Nef/Tat/Rev, Vpr/Vpu/Vif were measured at the last time point (yr 4-15; median 7 yr of ART).

Results: HIV Ab levels and avidity declined with time on ART and were positively associated with HIV DNA at yr 1, 4 and 4-15 of ART (r=0.35 and 0.38, respectively, p<0.001 at the last time point). Nef/Tat/Rev-specific T cell responses, but not responses against other antigens, correlated with HIV DNA levels (r=0.23, p=0.03). Neither Ab levels nor T cell responses correlated with cell-associated HIV RNA or plasma RNA by single copy assay. HIV Ab and avidity correlated with T cell responses to HIV Pol (r=0.3, p=0.01 and r=0.26, p=0.04, respectively) and to Nef/Tat/Rev (r=0.35; p=0.005 and r=0.39, p=0.001). There were no correlations between HIV Ab and T cell responses to HIV Gag or Env, or to CMV/EBV controls.

Conclusions: In individuals on ART, Ab to ENV/p24 and T cell responses to Nef/Tat/Rev correlate with each other and with HIV DNA levels but not with CA HIV RNA or residual plasma viremia. These findings suggest that the total frequency of HIV-infected cells (HIV DNA) may be a better marker of antigen expression that drives immune responses on ART than CA RNA in blood or residual viremia, which reflect activity of only a small fraction of proviruses that can be induced to express antigen. These results suggest the immune system is sensing infected cells; tracking immune responses may be a method of assessing the impact of reservoir-reducing strategies.

PP 5.2

Diverse interferons restrict HIV-1 Infection in macrophages through activation of SAMHD1

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Background: Macrophages are susceptible to HIV-1 infection despite abundant expression of a variety of anti-viral host proteins, perhaps most importantly the restriction factor sterile alpha motif domain and histidine/aspartic-acid domain-containing protein 1 (SAMHD1). SAMHD1 is subject to regulation by multiple cyclin-dependent kinases, which inactivate the enzyme by phosphorylation at a specific threonine residue (T592). We investigated the role of SAMHD1 and its phospho-dependent regulation in the context of HIV-1 infection in primary human monocyte-derived macrophages and the ability of various interferons (IFN) and pharmacologic agents to modulate this process.

Methods: CD14+ monocytes were isolated from whole blood of healthy donors and differentiated for seven days in media containing 10% pooled human serum. Mature, adherent macrophages were incubated with various interferons or pharmacologic agents, then analyzed for SAMHD1 protein and T592 phosphorylation or infected with HIV-1. To determine the role of SAMHD1-mediated, IFN-induced HIV-1 restriction, cells were treated with exogenous deoxynucleosides or virus-like particles containing the SIV protein, Vpx.

Results: Here we show that stimulation by Type I, Type II, and Type III interferons converge upon activation of SAMHD1 via dephosphorylation at Threonine-592 and do not exert their effect through changes in SAMHD1 protein levels. The IFN-induced anti-viral state was phenocopied by incubation with Dasatinib, a tyrosine kinase inhibitor, which resulted in dephosphorylation of SAMHD1. The viral restriction enforced via interferons or Dasatinib could be overcome by either incubation with excess deoxynucleosides or by addition of the SIV accessory protein Vpx in trans. Furthermore, we show that the levels of SAMHD1 phosphorylation determine macrophage susceptibility to infection in the absence of stimuli.

Conclusions: The results of this study show that SAMHD1 activation, and not protein induction, is the major effector function induced by Types I, II, and III IFN signaling in macrophages, and presents a pharmacologically actionable target through which HIV-1 infection can be subverted. This study resolves a longstanding question regarding the mechanisms behind SAMHD1-dependent IFN-induced HIV-1 restriction in non-cycling cells, such as macrophages. Here, we propose a common mechanism through which IFNs exert their potent anti-viral effects, and provide an explanation for the extreme donor-to-donor variability of macrophage susceptibility to HIV-1.

PP 5.3

TCF-1 expression is associated with HIV-specific CD8+ T cell proliferative capacity

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Background: Many HIV cure strategies propose to elicit HIV-specific CD8+ T cell responses to control and/or eradicate the virus, but they will not be effective if they do not prevent or reverse CD8+ T cell exhaustion. A loss in proliferative capacity is a key feature of exhaustion, but little is known about how this capacity is regulated in these cells. The purpose of this study was to explore the connection between the transcription factor TCF-1 and proliferative capacity in HIV-specific CD8+ T cells.

Methods: Cryopreserved PBMCs were sampled from Viremic (VL>8,000 copies/mL; n=14), ART-suppressed (VL<40 copies/mL on ART >2 years; n=10), and Controller (VL<40 copies/mL not on ART; n=12) HIV-infected individuals. Using flow cytometry, HIV-specific CD8+ T cells were identified by staining with Gag, Pol, or Nef-specific MHC Class I (HLA-A*02, *03, *24, or -B*07)-restricted tetramers. Tetramer+ cells were characterized for the expression of transcription factors (TCF-1, Tbet), effector molecules (Granzyme B), and surface proteins (PD-1, CD127, CCR7). Proliferation of the tetramer+ population was measured after 6-day *in vitro* peptide stimulation of CellTraceViolet (CTV)-labeled cells.

Results: HIV-specific tetramer+ CD8+ T cells from Controllers versus Viremic individuals had greater proliferative responses (%CTVlo 89% vs. 16%; p=0.05), were more likely to express CD127 (26% vs. 7%; p=0.0001), and were less likely to express PD-1 (60% vs. 95%; p<0.0001). Median TCF-1 expression was highest in tetramer+ cells from Controllers, followed by ART-suppressed and Viremic individuals (62% vs. 51% vs. 35%; p<0.0001). TCF-1 expression in these cells was associated with higher CCR7 and CD127, and lower PD-1, Granzyme B, and Tbet expression. Expression of TCF-1 (but not PD-1) strongly correlated with proliferative capacity amongst Viremic and Controller individuals (r=0.83, p=0.0008), and was inversely correlated with HIV VL in Viremic individuals (r=-0.85, p=0.02).

Conclusions: TCF-1 expression marks subpopulations of less terminally differentiated tetramer+ HIV-specific CD8+ T cells whose abundance correlates with enhanced proliferative capacity. Whether preservation of TCF-1+ cells is required to prevent HIV-specific T cell exhaustion remains to be investigated, but these data provide a rationale for future studies to evaluate TCF-1 as a target to enhance the efficacy of CD8+ T cell-based HIV cure strategies.

PP 5.4

Myeloid-derived suppressor cells decrease T-cell responses to viral antigens and therapeutic conserved elements DNA vaccine and increase following analytic treatment interruption

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Background: Therapeutic immunization with a DNA vaccine expressing conserved elements (CE) can redirect and broaden T-cell responses towards conserved regions of virus. However, immune suppression may limit the efficacy of such responses. Myeloid-derived suppressor cells (MDSC) are a suppressive subset of immature myeloid cells activated and expanded during inflammatory cytokine elevation. Best characterized in cancer, MDSC decrease anticancer T-cell responses. MDSC are now well characterized in HIV infection where they inhibit anti-viral T-cell responses. While antiretroviral therapy (ART) contracts MDSC, they persist despite viral control. We hypothesized MDSC suppression of antiviral T-cell responses decreases efficacy of CE-vaccination.

Methods: To determine the impact of MDSC in immunotherapy, we first examined the kinetics of monocytic mMDSC and granulocytic gMDSC frequency and function in SIV-infected rhesus macaques receiving ART alone (n=7) or in combination with therapeutic CE DNA vaccine (n=4). MDSC frequencies and suppression were analyzed by flow cytometry in fresh PBMC at various timepoints before, during and after SIV infection, ART, CE-vaccination and analytic treatment interruption (ATI). CE responses were measured by ELISpot.

Results: In 7 animals receiving ART without CE-vaccination, we observed elevated frequencies of suppressive gMDSC during SIV infection and ART and interestingly, a marked increase after ATI correlating with inflammatory cytokines *(Dross et al., JI, 2017)*. In 4 CE-vaccinated animals, the animal with the lowest median gMDSC frequency developed the highest CE-responses following vaccination and lowest viral loads at ATI. This suggests elevated gMDSC during HIV infection and ART may compromise immunogenicity of therapeutic vaccination. Additional studies are in progress to evaluate gMDSC in animals receiving CE-vaccination in combination with T-cell protection strategies, latency reversal and exhaustion blocking agents.

Conclusions: Our results to date suggest that gMDSC can suppress T-cell responses to viral antigens *in vivo* and reduce the efficacy of therapeutic vaccines. Furthermore, the remarkable increase in gMDSC following ATI is a concern as these responses may limit efficacy by suppressing the ability of vaccine-induced T-cell responses to control viral rebound. These studies suggest gMDSC may be a key factor in HIV cure strategies and new approaches that limit or reduce gMDSC frequency may be needed to achieve an HIV cure.

PP 5.5

SIV-specific CD8 T cells are largely excluded from B cell follicles during early SIV infection

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Background: While HIV- and SIV-specific CD8 T cell responses are critical for suppression of virus producing cells, these cells fail to fully suppress viral replication. During chronic infection, one potential mechanism for this failure is relatively low levels of virus-specific CD8 T cells in B cell follicles, where virus is most concentrated, permitting ongoing virus replication. It is not known whether this phenomenon also occurs during early infection. Here, we determined the location, abundance, and phenotype of SIV-specific CD8 T cells in lymph nodes from SIV-infected rhesus macaques during early infection.

Methods: The location, abundance, and phenotype of SIV-specific CD8 T cells were determined in lymph nodes via in situ MHC-class-I tetramer and immunohistochemical staining of nine SIVmac 239 infected Mamu A*01 rhesus macaques at 21 dpi.

Results: We found that levels of SIV-specific CD8 T cells in B cell follicles were significantly lower than in extrafollicular regions. Within follicles, SIV-specific CD8 T cells were largely excluded from germinal centers. A median of 62% of follicular SIV-specific CD8 T cells expressed PD-1. In addition, a median of 12% of follicular SIV-specific CD8 T cells were in direct contact with Foxp3+ cells, and 4% were themselves Foxp3+. Ki67 was expressed by a median of 41% of follicular SIV-specific CD8 T cells and 25% expressed high levels of perforin.

Conclusions: These data suggest that during early stages of infection, low levels of follicular SIV-specific CD8 T cells may permit ongoing viral replication, similar to what we previously reported in chronic disease. Furthermore, these data suggest that follicular SIV-specific CD8 T cells are possibly exhausted, that subsets may be inhibited by Foxp3+ Tregs, that subsets are activated and dividing, and subsets express the effector molecule perforin suggesting cytolytic potential. These findings provide important insights into SIV immunopathogenesis and may help inform future cure strategies.

PP 5.6

CD8 T cells from HIV positive individuals on ART have a skewed differentiation phenotype and impaired proliferative responses

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Background: Combination anti-retroviral therapy (cART) is effective in maintaining low viral loads, restoring CD4 T cell counts, and prolonging life.However, even individuals with durably suppressed viral load under cART experience residual immune dysfunction.CD8 T cells can detect and kill virally-infected cells and could be harnessed as part of a 'shock-and-kill' HIV cure strategy.We investigated the degree to which CD8 T cell function is restored in individuals on long-term cART.

Methods: We compared the phenotype and function of total and antigen-specific CD8 T cells between HIV+ individuals with durable viral suppression on cART ('HIV+ cART'; HIV RNA <50 copies/ml for >2 years) and HIV-seronegative individuals.We assessed antigen-specific cytokine production and degranulation (IFN-γ, TNFα, MIP-1β, Perforin, CD107a), antigen-specific proliferation, exhaustion (PD-1, CD160, Tim-3), and effector differentiation (T-bet and Eomesodermin).

Results: CD8 T cells from HIV+ cART and HIV-seronegative participants exhibited comparable capacity to secrete multiple cytokines and degranulate in response to antigen stimulation, and expressed exhaustion markers at similar frequencies.In HIV+ cART participants, PD-1 expression did not differ consistently between HIV- and CMV-specific CD8 T cells.However, relative to HIV-seronegative participants, memory CD8 T cells from HIV+ cART participants were skewed toward a T-bet^{high} Eomes^{low} effector-like phenotype, suggesting that these cells may not differentiate fully into memory cells.Supporting this hypothesis, memory CD8 T cells from HIV+ cART participants proliferated less robustly in response to antigen.In a preliminary analysis (n=3), IL-15 supplementation boosted proliferation of CD8 T cells from HIV+ cART participants.

Conclusions: Though some CD8 T cell function is restored under cART, cells retain an effector-like phenotype and exhibit impaired antigen-specific proliferation.Measures to ameliorate this residual dysfunction, such as IL-15 therapy, could be used to boost HIV cure and therapeutic vaccination strategies.

PP 5.7

Functional profiling of HIV-specific CTL clonotypes and their ability to reduce HIV reservoir

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Background: The success of the shock and kill strategy depends both on the reactivation of the latent reservoir and on the ability of cytotoxic T cells (CTLs) to recognize and kill HIV infected cells. Although several agents efficiently reactivate latently infected cells *in vitro*, they do not induce a measurable decrease of the HIV reservoir *in vivo* and are limited by systemic toxicity. Studies on the functional quality of CTL clonotypes are needed to elucidate what characteristics are required to eliminate the HIV reservoir.

Methods: We expanded Nef and Gag-specific CTL clones to characterize their functional qualities. We sequenced their TCR alpha and beta CDR3s to select different clonotypes and characterized them according to: i) antigen sensitivity by IFN-γ staining after stimulation at different peptide concentrations; ii) CD8 binding dependency by blocking the co-receptor with anti-CD8 antibody; iii) killing capacity by co-culturing the clones with peptide-pulsed EBV transformed B (B-EBV) cells; iv) capacity of CTL clonotypes to eliminate reactivated latently HIV-infected cells by HIVE assay.

Results: The 14 distinct CTL clonotypes tested were all cytotoxic (CD107a$^+$) and able to kill cognate peptide pulsed B-EBV cells. The antigen sensitivity measured by IFN-γ production spanned a range of 2 orders of magnitude (EC_{50} from 0.004 to 0.4) and correlated with killing capacity measured in lytic units (LU_{30} from 158 to 550) in our B-EBV assay, but lower antigen sensitivity was significantly associated with higher killing capacity. CD8 blocking did not completely inhibit degranulation, but abrogated IFN-γ production in all clonotypes, except one. While most of the CTL clones were not able to reduce cell-associated HIV DNA from infected CD4 cells, the single CD8 independent clone showed reduction, suggesting superior functionality.

Conclusions: We found that the CD8 independency, indicative of TCR affinity for the MHC/peptide complex, is associated with a reduction of HIV DNA in contrast with antigen sensitivity or lytic units. Characterizing the function of different CTL clonotypes might help define parameters indicative of efficient reservoir killing *in vivo*.

PP 5.8

Persistence of CD4+PD-1 high T cells despite long-term suppressive ART

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Background: CD4$^+$ T-cells expressing the checkpoint inhibitor PD-1 may be enriched for HIV infection, but the impact of ART on their frequency and association with measures of HIV persistence are uncertain.

Methods: We evaluated longitudinal changes in T-cell PD-1 expression and their correlation with HIV persistence and immune activation in ACTG A5321 participants who initiated ART during chronic infection and had well-documented sustained viremia suppression. We gated on two T-cell populations: positive for PD-1 (PD1$^+$) vs high expression (PD1^{hi}) since the latter subset is reported to have a different functional profile.

Results: Among the 97 participants, there were modest decreases to 1 year of ART in %PD1$^+$ (median 49.5% to 46%) and %PD1hi (median 0.7% to 0.4%) CD4$^+$ T-cells ($p<0.001$; signed rank); the relative reduction in CD4$^+$PD1^{hi} T-cells was substantially greater than CD4$^+$PD1$^+$ T-cells (median 43% vs 9%; $p=0.01$). %CD4$^+$PD1$^+$ T-cells continued to decrease to year 4 and to 6-15 years on-ART while %CD4$^+$PD1^{hi} T-cells only declined to year 4. Pre-ART %CD4$^+$/CD8$^+$PD1$^+$ correlated strongly with frequencies on-ART (Spearman r=0.92-0.95), while correlations for PD1^{hi} expression were not as strong (r=0.28-0.52). %CD4$^+$PD1^{hi} levels positively correlated with cell-associated HIV-1 DNA pre-ART (r=0.22) and at 1 (r=0.25) and 4 years (r=0.24) on-ART, but %CD4$^+$PD1$^+$ levels after ART initiation did not. Residual viremia, measured by single copy assay, ≥4 years on-ART did not correlate with PD1$^+$/PD1^{hi} populations. Similarly, no correlations were observed with cell-associated HIV-1 RNA. %CD4$^+$PD1^{hi} consistently correlated with CD4$^+$ T-cell activation (%HLA-DR$^+$CD38$^+$) at years 1 (r=0.40; $p<0.001$) and 4 (r=0.43; $p<0.001$) on-ART but not pre-ART. %CD8$^+$PD1^{hi} strongly correlated with %CD4$^+$PD1^{hi} at all timepoints (r=0.80-0.92) but did not consistently correlate with HIV persistence measures or T-cell activation. %CD4$^+$PD1$^+$ didn't correlate with T-cell activation at any timepoint.

Conclusions: PD-1 expression on CD4$^+$ or CD8$^+$ T-cells on-ART is most strongly associated with pre-ART expression indicating long-lasting effects despite HIV-1 suppression. Among T-cells expressing PD-1, the CD4$^+$PD1^{hi} subset stabilized after initially declining on ART and was positively associated with HIV DNA levels and CD4$^+$ T-cell activation, whereas other CD4$^+$PD1$^+$ T-cells were not. Distinguishing

CD4+ T-cell subsets by levels of PD-1 expression may be important for targeting the HIV reservoir.

PP 5.9

Novel dual role of dendritic cells in priming de novo CTL responses while inhibiting memory CTL responses to HIV-1 through the PD-L1 pathway

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Background: Eliciting highly functional, primary cytotoxic CD8 T cell (CTL) responses to a broad range of epitopes is likely required for CTL to eliminate the chronic HIV reservoir. However, the combination of CTL exhaustion and the establishment of CTL escape variants represent major hurdles towards this goal.Here we reveal a new, unique role of T helper cell factor CD40L and the immune checkpoint PD-1 pathway in type-1 programmed DC (DC1) selectively driving highly functional de novo CTL responses from naïve rather than memory T cells.

Methods: Expression of PD-L1, the ligand for PD-1, was evaluated on differentially activated DC before and after CD40L signaling. We compared the ability of HIV antigen-loaded DC1 in the presence CD40L 'help', and anti-PD1 blocking antibody (Ab) to induce, HIV-specific CTL from naïve or memory CD8 T cells from chronic HIV-infected MACS participants.

Results: Immature DC constitutively expressed low levels of PD-L1, while PD-L1 expression on DC1 was substantially higher. PD-L1 expression on DC1 was further enhanced upon CD40L stimulation.Moreover, the presence of CD40L improved DC1-mediated priming of naïve CD8 T cells, but inhibited memory CD8 T cell responses. Because this dual function of promoting primary while inhibiting memory CD8 T cell responses could be related to the impact of CD40L on PD-L1 expression, we tested the effect of PD-1 blocking Ab on induction of CTL responses by DC1. Importantly, PD-1 blockade inhibited the priming, activation and differentiation of naïve CD8 T cells into effector CTL. Naïve T cells could differentiate into effector $Tbet^{hi}/Eomes^{low}$ only when anti-PD1 was added upon restimulation. In contrast, PD-1 blockade enhanced the overall magnitude of memory HIV-1-specific CTL responses and reversed the exhausted memory phenotype from $Eomes^{hi}/T\text{-}bet^{low}$ to a $Tbet^{hi}/Eomes^{low}$ phenotype.

Conclusions: Our data support novel, important roles for both CD40L and PD-1 in DC1-mediated induction of primary versus memory CTL responses to HIV-1. This new, enhancing effect of PD-1 on priming of anti-HIV CTL should be considered in use of anti-PD1/PD-L1 Ab therapies in HIV infection. Exploiting this unique programming of CTL by DC could be important in clinical therapies aimed at eliminating the HIV reservoir.

PP 5.10

Intrinsic resistance of HIV-infected macrophages to CTL-mediated killing drives immune activation

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Background: CD4+ T lymphocytes are the principal target of HIV infection, but macrophages can also become infected and contribute to viral pathogenesis. CD8+ cytotoxic T lymphocytes (CTLs) control virus levels during acute and chronic stages of HIV infection and reduce HIV disease progression. Most studies have focused on CTL control of infected CD4+ T cells with less focus on infected macrophages. Recent work suggests that SIV-infected macrophages are relatively resistant to CTL-mediated killing, but the mechanism behind their differential susceptibility is unknown. Thus, the objective of this work was to characterize the interactions between CTLs and HIV-infected targets, both CD4+ T cells and macrophages, to delineate immunoevasion mechanisms of macrophage resistance to CTL-mediated elimination.

Methods: Monocytes were matured into macrophages while CD4+ T cells were activated to permit infection with HIV strain 89.6. Immunological techniques, including flow cytometry-based elimination assays and HIV Gag p24 ELISA-based suppression assays, were used to asses the susceptibility of autologous HIV-infected targets to CTL-mediated killing. Flow cytometry and ELISA-based recognition assays were used to characterize the CTL degranulation and cytokine response to the targets. Imaging flow cytometry was used to assess effector-target conjugates while cytokine-bead arrays characterized pro-inflammatory chemokines released by macrophages.

Results: We demonstrate that macrophages exhibit delayed CTL-mediated killing as compared to CD4+ T cells ($p<0.0001$), resulting in inefficient HIV suppression ($p=0.0005$). Mechanistic studies reveal that delayed killing of macrophages is caspase-3- and granzyme B-dependent, whereas rapid killing of CD4+ T cells is caspase-independent and does not require granzyme B. Moreover, impaired killing of macrophages is associated with prolonged effector-target contact time ($p=0.0019$) and greater CTL IFN-γ expression ($p=0.0047$), inducing macrophage production of pro-inflammatory chemokines that trigger recruitment of monocytes and T cells.

Conclusions: These results suggest that inefficient CTL-mediated killing of macrophages may contribute to reservoir persistence and chronic inflammation in HIV infection. An improved understanding of the precise mechanisms underlying the observed resistance to target cell killing and resulting hypersecretion of pro-inflammatory cytokines and chemokines will be necessary to develop approaches capable of efficiently eliminating infected macrophages and hampering chronic inflammation.

PP 5.11

Comparative transcriptome profiles in HIV-infected persons according to their clinical phenotype

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Background: HIV-infected people are not all equal towards HIV virus, at least regarding clinical and biological progression. Rather rare cases of individuals that do not progress or spontaneously control viral replication after a period of ART treatment are intriguing. Our objective was to compare different clinical phenotypes between them and with healthy donors using discriminating gene analysis, in order to identify family of genes that may distinguish them.

Methods: We performed a Gene Set Enrichment Analysis. Using curated gene sets from the Reactome Pathway Database, we detected upregulated gene sets in each of the two phenotypes. We performed hierarchical clustering of the patients to identify clusters of patients with similar gene expression profiles. Using classical multidimensional scaling, we projected the patients on four-dimensional space.

Results: We performed a pilot study including two treated post-treated aviremic seroconverters (DE, MEZI), three non treated chronic HIV-infected patients (H1 to H3), one viremic seroconverter (TW), one viremic patient that does not progress and consequently does not need any treatment (CD) and four healthy donors (DS1 to DS4), comparing their transcriptome profiles. After 20,000 gene set permutations and with a stringent cutoff with false discovery rate <5% we found 14 gene families upregulated in HIV+ patients and 87 gene families upregulated in HIV- patients. The four-dimensional projection shows a clear separation of gene expression profiles between negative controls and HIV+ who split again into two subclusters. The heatmap

expressing 20 upregulated genes in HIV+ and 20 genes in HIV- show clear differences between them, as well as differences between clinical phenotypes of HIV+ persons.

Conclusions: This work suggests correlates of immune protection induced by early treatment in specific profiles of HIV-infected patients through Transcriptome analysis. Our analysis may allow better understanding causes of non activation of the immune system, as well as innate factors contributing to viral control.

PP 5.12

Characterisation of immune exhaustion in natural killer cells and role in HIV infection

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Background: Expression of immune exhaustion markers in HIV specific $CD8^+$T cells has been associated to immune dysfunction and progression of infection. However, immune exhaustion can also be detected on other immune cells(eg. NK cells), although less is known about the functional consequences. Here we characterized the expression of exhaustion markers on NK cells during HIV infection and evaluated their role in NK cell function.

Methods: PBMC samples were selected from HIV-negative and HIV-infected donors, including samples from 12 HIV-positive donors prior to antiretroviral treatment (ART), and following ART suppression of viremia for ≥12 months. A flow cytometry panel was designed to analyze immune exhaustion markers including LAG-3, CTLA-4, PD-1, TIM-3 and TIGIT, along with lineage markers CD3, CD8 and CD56. NK cell function was measured as degranulation (CD107a) and IFN-γ production after culture with K562 cells.

Results: Cells from 15 HIV-negative donors, 22 HIV-infected individuals with suppressed viremia and 12 HIV-viremic donors were analyzed. CTLA-4 and TIM-3 expression was similar in NK cells from the three cohorts, while LAG-3 and TIGIT were more expressed in HIV-negative and HIV-suppressed than in HIV-viremic donors. On the contrary, PD-1 was upregulated in NK cells from viremic individuals. Interestingly, TIGIT and TIM-3 expression positively correlated with degranulation of NK cells after culture with K562 cells. Longitudinal analysis showed a significant increase in TIGIT expression after suppressing viremia with ART. Conversely, $CD8^+$T cell expression of PD-1, TIGIT and TIM-3 significantly decreased after ART, reaching HIV-negative donors values. Degranulation capacity of NK cells was similar among groups, while IFN-γ production after ART was similar to HIV-negative donors. There was no correlation of any of these markers in NK or $CD8^+$T cells with the size of the reservoir measured as IUPM.

Conclusions: NK cells from virally suppressed HIV patients have similar expression of markers of exhaustion, and similar function to that of HIV-negative donors, while these markers are altered in viremic donors. TIM-3 and TIGIT expression was associated with better function of NK cells, and thus further investigation should be conducted to evaluate if NK cells with upregulation of these markers have enhanced immunotherapeutic, antiviral activity in vivo

PP 5.13

CXCR3/CCR6 double positive germinal center T follicular helper cells (GC TFH) harbor residual virus during cART initiated during hyperacute HIV infection

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Background: Understanding the nature of the latent reservoir in tissue sanctuary sites is critical to the success of HIV cure strategies. A few human and non-human primate studies have reported that HIV-1 continues to replicate in lymph nodes (LN) during therapy while other studies have found no evidence of ongoing replication across multiple tissue compartments. A recent study identified CD32a as a marker for latently infected cells, but whether CD32a+ cells also harbor ongoing virus replication remains unknown. Here, we used excisional LN and paired blood samples from hyperacute HIV infected subjects who initiated cART in Fiebig stage I to determine if there is persistent virus replication and identify cell subsets that harbor residual virus during cART.

Methods: Flow cytometry was used to phenotypically define germinal center (GC) Tfh subsets. Immunofluorescence microscopy combined with in situ RNA hybridization 'RNAscope' techniques was used to phenotypically define and localize HIV-infected cells in LN. Furthermore, virus evolution was defined by comparing plasma-derived transmitted/founder viral sequences to LN–derived sequences after 6 months of cART.

Results: The expansion of GC Tfh was significantly attenuated in early treated subjects compared to chronic untreated subjects (p=0.01). Despite cART-mediated complete plasma viral suppression, HIV Gag p24 antigen was persistently detectable almost exclusively in the GCs. Majority of GC TFH exhibited high expression of CXCR3 and CCR6 and were peristently Gap p24 positive. Importantly, viral sequence evolution was observed in one early treated individual in whom we had plasma HIV sequences prior to cART initiation and LN HIV sequences after six months of suppressive cART.

Conclusions: Persistent detection of HIV-Gag p24 protein in $CXCR3^+CCR6^+$ GC TFH and the evidence of virus sequence evolution in LN suggests that there could be ongoing virus replication in LN tissues despite early initiation of cART at Fiebig stage I. HIV cure strategies should take into consideration the influence of potential ongoing virus replication in santuary sites during ART.

PP 5.14

Drug-induced modulation of cellular activation during latency reversal changes antigen processing and peptide presentation in primary CD4 T cells

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Background: Latency reversal agents (LRA) PKC agonists (PKCa) Bryostatin and Ingenol-B induce HIV re-expression but cause non-negligible cellular activation, triggering undesired cytokines release and proliferation. To abrogate these effects, immunomodulatory agents (IMA) like ruxolitinib or rapamycin have been used in combination with PKCa. Immune clearance of LRA-reactivated cells requires HIV peptide generation by the antigen processing machinery and presentation by MHC. The hydrolytic activities of the degradation machinery are tunable by various stimuli, causing changes in protein degradation. We hypothesized that PKCa- and IMA-treatment might alter antigen processing and MHC-peptide presentation in CD4 T cells.

Methods: We evaluated the effects of PKCa-, CD3/28- and IMA-treatment on the hydrolytic activities of peptidases using a fluorometric assay in live primary CD4 T cells and monitored cellular activation by flow cytometry. We assessed how these treatments affect antigen processing using in vitro degradation of synthetic HIV peptides in matched cytosolic extracts of PKCa-, IMA- or mock-treated CD4 T cells and defined the MHC-peptidome of PKCa- and mock-treated PBMC by mass spectrometry.

Results: While CD3/28-activation caused the highest increase in cytosolic peptidase activities that positively correlated with the percentage of activated CD4 T cells, PKCa-treatment significantly increased peptidase activities up to 4.9-fold but did not necessarily correlate with the proportion of activated cells. Treatment with IMA in combination with PKCa or CD3/28 reduced cellular activation and peptidase activities by up to 10-fold but did not return to the levels of mock-treated cells. Degradation of HIV peptides in cytosolic extracts revealed distinct changes in the degradation patterns upon CD3/28-, PKCa- or IMA-treatment. The generation of known epitopes and potential MHC-binders was affected in a drug- and sequence-dependent manner. Treatment with Bryostatin partly changed the

MHC-peptidome of PBMCs, showing common as well as some unique peptides coming from different locations within their source proteins.

Conclusions: Changes in peptidase activities and degradation patterns induced by PKCa or IMA are not necessarily linked to the activation status of the cells and are specific to the drug used. The modulation in antigen processing and peptide presentation caused by drug treatment diversifies the range of CD8 responses needed for clearance of reactivated HIV-infected cells.

PP 5.15

Impact of time of ART initiation on HIV specific T cell functionality in perinatally infected children

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Background: Early initiation of antiretroviral therapy (ART) in vertically HIV-infected children provides an opportunity to limit the size of reservoir, but whether and how the time of ART treatment initiation can durably impact host immune responses associated with HIV infection is still unknown. In this study, we analyzed HIV-specific CD4 T cell functionality in vertically HIV-infected children in whom ART was initiated early or late after birth.

Methods: PBMCs were collected from perinatally HIV-infected children (age range 9-15 yrs) with durable viral control (plasma HIV-RNA<50 cp/mL) enrolled at Bambino Gesù Children Hospital. In 5 Early Treated (ET, Age at ART 0-0.5 yrs), and 6 Late Treated (LT Age at ART 1-12 yrs) antigen-specific (CD40L+CD4+) T cell functionality was evaluated by flow cytometry for intracellular cytokines (IL2, IFNγ, TNFα, IL21) following 18 hr stimulation with gp140. Differences between groups were determined by Mann-Whitney t tests (P≤0.05). PLS-DA analysis were performed using the mixOmics package in R.

Results: Frequencies of gp140 specific CD4 T cells were not different between ET and LT. However ET showed a better quality of these cells demonstrated by a higher proportion of cytokine producing T cells compared to LT. In particular, ET were enriched in polyfunctional T cells, defined by the simultaneous detection of 2+ cytokines, dominated by IL2 production. On the contrary, LT response was paucifunctional. The different CD4 T cell profiles induced by gp140 stimulation in ET and LT was confirmed by PLS-DA analysis where these 2 groups were clearly separated.

Conclusions: Our results suggest that time of ART initiation in HIV-infected children has a long-term impact on the quality but not the quantity of the host HIV-specific T-cell immune responses and also reinforced the importance of early treatment initiation. Larger studies are warranted to confirm these characteristics of host immunity and whether they can be targeted in functional cure approaches.

PP 5.16

Persistence of antigen presenting cell-mediated HIV trans infection during cART

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Background: Professional antigen presenting cells (APC; dendritic cells [DC], macrophages, B cells) mediate highly efficient HIV trans infection of CD4 T cells. APC from HIV infected nonprogressors (NP) are inefficient mediators of HIV trans infection compared to progressors (PR) and seronegatives (SN). Efficient trans infection could contribute to HIV persistence during cART. We evaluated the ability of APC from NP and PR on cART to trans infect CD4 T cells, to better understand the role of cell-to-cell virus spread in HIV persistence.

Methods: HIV R5-tropic Bal-loaded APC from 5 NP and 5 PR on cART were mixed with autologous or heterologous (single HIV SN donor), activated CD4+ T lymphocytes to measure trans infection. APC from 5 SN exposed to cARTwere also tested for their ability to transfer virus to autologous CD4T cells in vitro. Virus replication was measured by p24 ELISA (NCI).

Results: APC from NPs and PRs were tested prior to and after cART initiation. To control for the effect of residual drug, APC trans infection was tested using both autologous and heterologous (SN) CD4 T cells. For all NPs, APC maintained their inability to trans infect CD4 T cells before or after cART. Strikingly, none of the PR tested showed impairment in trans infection either prior to or after initiation of cART. Moreover, CD4 T cells from PR resistant to HIV cis infection were susceptible to trans infection mediated by APC. APC from 5 SN exposed to 4 different classes of ARTat physiological concentrations in vitro maintained their ability to trans infect CD4 T cells, while cis infection of CD4 T cells was inhibited.Trans infections was only inhibited when APC and/or CD4 T cells were exposed to a CCR5 antagonist and DC were used as APC, but not when B cells were tested as APC.

Conclusions: The ability of APC from PR on cART to trans infect CD4 T cells suggests an essential role for APC–mediated trans infection in maintaining the HIV reservoir, perhaps through frequent interactions in lymphoid tissue with CD4 T helper follicular cells, which are primary targets for HIV. This mechanism could limit cART effectiveness.

PP 5.17

Preservation of IL-17 producing γδ T cells and their role in the control of immune activation in HIV controllers: ANRS EP56 study

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Background: Among γδ T lymphocytes, Vδ2+ cells were reported to be part of the HIV reservoir. CCR6+ T cells were shown to carry HIV-DNA. Preferential depletion of CCR6+ Th17 cells from the gut mucosa is associated with increased microbial translocation and chronic inflammation. γδ T cells also primarily reside in the gut and have been shown to share certain phenotypic and functional features with Th17 cells. The aim of the study was to analyze the characteristics of γδ T cells and their relationship with immune activation and HIV reservoir in HIV controllers (HIC).

Methods: PBMC were isolated from 17 HIC (median (IQR) CD4 count:773 (541-971);VL <50, HIV-DNA levels:1.6 log/million PBMC (1.3-2.2) and as controls, 17 patients with untreated chronic HIV infection (UT-CHI, median CD4 count: 494/ml; median VL: 4.2 log/mL) and 17 healthy donors (HD). Phenotype & cytokine production by γδ T cells were analyzed using flow cytometry. sCD14 and IP-10 were measured by ELISA. Spearman correlation tests were performed for statistical analyses with P<0.05 considered as significant.

Results: The proportion of γδ T cells (median: 4.8% (2.3-7.7)) positively correlated with total HIV-DNA levels (r=0.53, p=0.041), CD38+HLA-DR+CD8+ T cells (r=0.53, p=0.002) and with plasma levels of IP-10 (r=0.55, p=0.013).The proportion of Vδ2+ cells was reduced in HIC compared to HD (p=0.011). Interestingly, the proportion of IL-17+ γδ T cells was preserved in HIC (1% (0.5-1.6) vs 1.2% (0.6-2.8) in HD) in contrast to UT-CHI (0.2% (0.1-0.4);p=0.0002 vs HD). Plasma levels of sCD14 and IP-10 were higher in HIC compared to HD. There was a strong relationship between IP-10 levels and CD8 T-cell activation (r=0.55, p=0.001). The proportion of IL-17+γδ T cells negatively correlated with plasma levels of IP-10 (r=-0.37, p=0. 039) and sCD14 (r=-0.39, p=0.027).

Conclusions: The relationship between the frequency of γδ T cells and both levels of total HIV DNA and immune activation suggest that this T-cell subset may contribute to HIV persistence. In the other hand, the preservation of IL-17+γδ T cells in HIC and their negative association with immune activation suggest that IL-17+γδ T cells participate in the control of chronic T-cell activation in HIV controllers.

PP 5.18

Defining the landscape of HIV-specific T-cell responses in HIV-1 infected durably suppressed participants

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Background: There is no cure for HIV-1, largely because HIV establishes a small but sustained pool of latently infected cells that are not cleared by antiretroviral therapy. We and others are investigating strategies to firstly reactivate the latent HIV reservoir and then use T cell immunotherapy to clear reactivated cells. However, the presence of pre-existing T cell escape variants in the reservoir may limit CD8+ T cell recognition of HIV, and therefore the clearance of the reactivated cells. Currently, the level of pre-existing virus escape in the reservoir is unclear. In order to design effective T cell immunotherapies to boost and, or induce de novo T-cell responses, we investigated the landscape of T cell responses in durably suppressed HIV-1 infected participants.

Methods: HIV-1-specific T cell responses in HIV-1 infected durably suppressed (average 5.5 years) participants were examined both cross-sectionally and longitudinally (weekly, monthly and yearly). T cell responses were measured against either overlapping peptides spanning the HIV-1 clade B consensus proteome, or previously defined optimal CD8+ T cell epitopes (hiv.lanl.gov) using ex vivo IFN-g ELISpot.

Results: Despite long-term viral suppression, HIV-1 specific T cell responses were maintained and robust (~2000 SFU /106 PBMC). T cell responses were also remarkably stable (median CV=16% range 4-31%). Power calculations derived from these data suggest that group sizes as low as six are sufficient to examine the immunogenicity of T cell vaccines.

We next mapped HIV-1 specific T cell responses in HIV-1 durably suppressed participants against the HIV-1 clade B consensus proteome using ex vivo IFN-g ELISpot. In parallel, replication competent viruses derived from supernatants of autologous resting CD4+ T cells following mitogenic reactivation were sequenced using SMRT methodology to perform HIV Half Genome Sequencing, and the presence of escape variants were experimentally confirmed. We detected a wide (1-18 responses) breadth of T cell responses across our cohort, with all HIV-1 proteins targeted. We also identified escape variants in the latent reservoir.

Conclusions: On-going studies are focusing on estimating the level of pre-existing escape and examining patterns of escape in the reservoir, which in turn will inform the design of T cell immunotherapies.

PP 5.19

Multiple NF-κB elements in the LTR of HIV-1 subtype C coordinate with the auto-regulatory circuit of Tat to drive rapid establishment of latency

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Background: The LTR of HIV-1 subtype C (HIV-1C) contains three or even four NF-κB motifs that confer superior transcriptional activity on the viral promoter. How the enhanced transcriptional strength of the promoter-variant strains of HIV-1C modulates viral latency is the primary aim of the present study.

Methods: We engineered three different Jurkat cell models to examine latency in HIV-1C. The autonomous Tat-feedback (ATF), the disjoint Tat-feedback (DTF) and the tuneable Tat-feedback (TTF) models differ from one another based on the presence and flexibility of the LTR-Tat feedback axis. We generated panels of reporter viral strains under each of the models expressing GFP under the C-LTR. The pseudotyped reporter viral strains in each panel are genetically identical except for the variable number of functional NF-κB motifs ranging from 4 to 0 copies. Stably infected GFP^{+ve} Jurkat cell pools or cell lines were generated to monitor reporter expression profiles using flow cytometry. The array of host factors recruited at the active (GFP^{+ve}) and latent (GFP^{-ve}) promoters was assessed using ChIP. Statistical evaluation was performed by 2-way ANOVA.

Results: The ATF model identified a positive correlation between the copy-number of NF-κB motifs, transcriptional strength of the promoters and the rapidity with which latency was established. In the DTF model, latency establishment was independent of NF-κB copy-number under normalised Tat concentration. In the TTF model, a temporal sequence of silencing events was evident for a stronger (3-κB) but not a weaker (1-κB) viral promoter in a Tat-dependent manner. ChIP assay in the ATF model revealed a reciprocal binding of p50, p65, NFAT1 and NFAT2 at the active and latent promoters.

Conclusions: Our data suggested that the transcriptional strength of HIV-1C LTR is an important parameter regulating viral latency. Additionally, silencing of the strong promoters (4- and 3-κB LTRs) may be regulated differently at the molecular level. Furthermore, a positive correlation between the Tat transcript levels and the rapidity of GFP switch off in the stronger viral promoters is indicative of the Tat-mediated positive feedback playing a critical role in regulating viral latency.

Session 6: Human studies

OP 6.1

HIV-seroreversion dynamics after allogeneic stem cell transplantation

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Background: Allogeneic stem cell transplantation (allo-SCT) in HIV-infected subjects with severe hematological malignancies is the only described strategy capable to dramatically reduce HIV latent reservoir. Whether this putative eradication strategy is associated with seroreversion has not been established yet. Within the IciStem consortium, we explored the longitudinal serostatus of HIV+ individuals after allo SCT.

Methods: Longitudinal plasma samples from 13 HIV+ allo-transplanted patients under cART were analyzed. HIV-1 serostatus was tested in a qualitative western blot assay (New Lav Blot I, Biorad). For 7 subjects with longer follow-up (>2years) additional analysis was done using the standard and low-sensitive (LS) versions of the VITROS anti- HIV-1 assay (Ortho-Clinical Diagnostics) and the LAg avidity assay.

Results: Evolution of the HIV-specific antibodies in plasma was studied for 13 allo-SCT patients, all of them under cART. We observed that p24 and/or p31 disappeared in 9/13 patients, sometimes only three months after allo-SCT. gp140, gp160, and gp120 bands persisted in most individuals. Surprisingly, in two cases we found an undetermined (Pt#19 and Pt#28) western blot. LAg avidity assay was negative in 6/7 individuals with longer follow. LS-VITROS detuned assay showed that transplanted patients presented lower antibody levels than viremic and successfully suppressed HIV+ controls. These levels started to decrease directly after allo-SCT. Remarkably Pt#19 and Pt#28 presented antibody levels close to HIV negative donors.

Conclusions: We conclude that allo-SCT not only remarkably decreased the HIV latent reservoir but also reduced the level of HIV antibodies in presence of cART. We have observed evidence of seroreversion a few years after allo-SCT. Future cART discontinuation will unravel the role of the antibodies dynamics in the HIV cure.

OP 6.2

Sequencing HIV proviruses over time provides new insights into reservoir decay

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Background: At the initiation of ART, the viral load rapidly declines to very low levels, but integrated HIV DNA measurements show only minor changes over time, suggesting the reservoir is relatively stable.However, DNA measures may mask dynamic changes in thereplication-competent reservoir due to the large excess of defective proviruses. Herein, we investigate the dynamics and character of HIV proviruses for up to 9 years after ART initiation.

Methods: We sequenced HIV proviruses from 5′ LTR to 3′ LTR, capturing nearly the full length of the HIV genome, from two HIV-infected individuals at four time points after initiating ART. Greater than 800 proviruses were PCR-amplified at limiting dilution, purified, and sequenced on a MiniSeq (Illumina). Proviral integrity was determined through annotation of viral ORFs and stem loops.

Results: In patient 1, the fraction of intact proviruses decreased over time on ART (p<.002 first versus last time point). The decay appeared exponential with a half-life of 3.0 years, exponential decay rate –0.23/year, trending different than 0, p=0.08.In patient 2, a very similar pattern of viral decay was seen at the first 3 time points (half-life of 2.1 years); however, at the last collection, there was a remarkable increase in intact proviruses.This was followed by a few episodes of low level viremia on ART (peak of 60 copies/ml). Meanwhile proviruses with 0 intact open reading frames either remained the same or increased over time.

Conclusions: Our data suggests that sequencing proviruses longitudinally provides an alternate method to measure reservoir decline. Notably, intact proviruses appear to decay faster than defectives but occasional episodes of reservoir expansion can affect overall decay. An indirect but important implication of our study is that a majority of the reservoir is expressed over time.This is supported by the decline of intact proviruses, 85% in patient 1 over 88 months, and 72% in patient 2 over 48 months, while there was no decline in proviruses with 0 intact ORFs in the same patients. Understanding the mechanisms responsible for the observed decline as well as why and when reservoir expansion occurs will be important for HIV eradication.

OP 6.3

Brief ATI does not alter the size or composition of the latent HIV-1 reservoir

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Background: The effect of an analytical treatment interruption (ATI) on the latent reservoir remains unknown. We evaluated the impact of transient viremia on the reservoir in participants who underwent ATI in A5340, a clinical trial investigating the effect of VRC01 during ATI. We also assessed the relatedness of viruses sampled pre-ATI and at rebound.

Methods: We quantified total HIV-1 DNA and cell-associated RNA in CD4+T cells and replication competent virus by quantitative virus outgrowth assay (QVOA) in resting memory CD4+T cells from leukopharesis samples collected pre-ATI and 6 months post-ART resuppression in 9 participants. Single genome sequencing-derived gp160 env sequences from plasma virus obtained pre-ART, at rebound, and from pre- and post-ATI QVOA cultures were analyzed phylogenetically. Select Envs were tested for VRC01 neutralization sensitivity.

Results: Participants median ART duration prior to ATI was 4.7 years (range 3.6–14.5). The median duration of viremia during ATI was 5 weeks (range 4–6) and participants were suppressed on ART for a median of 34 weeks (range 23–44) prior to post-ATI sampling. Total DNA, cell-associated RNA, and infectious units per million cells by QVOA were not statistically different pre- and post-ATI (P>0.3, Wilcoxon signed rank test), with median log10 change of 0.3 copies, 0.08 copies, and –0.05 infectious units per million cells, respectively. In each participant, pre- and post-ATI QVOA sequences fell within

the pre-ART plasma phylogeny, but did not specifically align within rebound lineages. Expanded clones comprised 30% to 95% of participants' reservoirs, with similar frequencies pre- and post-ATI. Thus, sequences showed no evidence for enrichment of rebound viruses post-trial and pre-trial QVOA viruses failed to predict the identity of rebound virus. Pre-ART, rebound and QVOA Envs for each participant had similar IC50s to VRC01.

Conclusions: Quantitative and phylogenetic analyses suggest a brief ATI does not expand the latent reservoir. While clonal QVOA populations comprised a substantial fraction of replication competent peripheral latent virus, they did not rebound in vivo upon ATI despite similar VRC01 sensitivities. Results provide reassurance for participants of clinical trials employing ATI and highlight the challenge of accurately characterizing the full range of the replication competent latent reservoir that reactivates in vivo.

OP 6.4

No residual virus replication in a randomised trial of dolutegravir intensification

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Background: Whether residual virus replication (RVR) persists in HIV-infected individuals on suppressive antiretroviral therapy (ART) remains controversial. One strategy used to demonstrate RVR is intensifying ART with an integrase inhibitor and measuring increases in 2-long terminal repeat (2-LTR) circles. Previous studies with raltegravir demonstrated RVR in a subset of ART-suppressed individuals. Here we investigated the effects of dolutegravir.

Methods: In a randomised, placebo-controlled, double-blinded clinical trial, HIV-infected adults with virological suppression for >3 years were randomly assigned 1:1 to dolutegravir 50 mg or placebo daily for 56 days in addition to background ART. The primary outcome measure was the level of 2-LTR circles in CD4+ T cells at day 7. Cell-associated unspliced (CA-US) HIV RNA, total and integrated HIV DNA, and plasma HIV RNA using a single copy assay (SCA) were quantified by real-time PCR;T cell expression of HLA-DR, CD38 and PD-1 by flow cytometry, and plasma levels of interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP), d-dimer and soluble CD14 (sCD14) by ELISA. We used repeated-measures analysis of variance (ANOVA) as the protocol-defined primary analysis. Student's t-test or rank sum test, were used to compare changes from baseline to specific time points across study arms.

Results: We enrolled 40 HIV-infected individuals; 21 were allocated to dolutegravir and 19 to placebo with 14 and 11% receiving a protease-inhibitor based ART regimen respectively. All participants completed the study. There was no significant difference in the primary endpoint, 2-LTR circles in peripheral blood CD4+ T cells, as assessed by repeated-measures ANOVA over 7 days (p=0.17) or any other time point. Median (IQR) 2-LTR circles fold-change from baseline to day 7 was –0.17 (–0.90 to 0.90) in the dolutegravir and –0.26 (–1.00 to 1.17) in the placebo groups. We found no consistent difference in the levels of CA US HIV-RNA, total and integrated HIV DNA (Figure), SCA, T cell activation markers or plasma levels of sCD14, d-dimer, IL-6 or hs-CRP. PD-1 expression in CD4+ T cells declined slightly after 56 days in placebo recipients compared to dolutegravir (p=0.03).

Conclusions: In a randomised, double-blinded, placebo-controlled trial of dolutegravir intensification, there was no evidence of RVR on ART.

OP 6.5

A phase 2 trial to evaluate the effects of 3BNC117 in addition to antiretroviral therapy on the latent reservoir and viral rebound

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Background: Analytical treatment interruption (ATI) leads to rapid viral rebound in HIV-1 infected patients. Infusions of the bNAb 3BNC117 are associated with delayed viral rebound after ATI. In this study, we aimed to characterize the impact of 3BNC117 infusions in the presence of ART on the latent reservoir and on ATI.

Methods: 15 participants on suppressive ART not screened for 3BNC117 sensitivity received four 30 mg/kg intravenous infusions of 3BNC117 on day 0, week 12, week 24 while on ART. ATI was initiated 2 days after the third 3BNC117 infusion, and patients received a fourth 3BNC117 infusion at week 27. Leukapheresis was performed 2 weeks before the first infusion and at week 23, 1 week before ATI. Quantitative and qualitative viral outgrowth assays (Q^2VOA) were performed for 10 participants with paired leukapheresis samples. SGA was performed on plasma rebound viruses. Neutralization was assessed by TZM-bl assay.

Results: No significant changes were observed in the size of the latent reservoir after 2 doses of 3BNC117 in patients on suppressive ART. Phylogenetic analysis of the latent viruses obtained by Q^2VOA (623 viruses, ~34 per time point) showed significant changes in frequency of individual latent viral clones over the 23 week observation period in 3 of 10 participants, including contractions and expansions, but there was no correlation between the changes in clone frequency and sensitivity to 3BNC117. Following ATI, we observed a median time to rebound of 5.5 weeks, ranging from 2 to 17 weeks, in participants with baseline sensitivity to 3BNC117. Analysis of the emerging rebound viral sequences and neutralization profiles showed no apparent resistance to 3BCN117 in 6 out of 10 participants. Additionally, Q^2VOA was able to identify latent viruses that were highly similar (96.8 to 100% similar) to the rebound viruses in all 10 patients.

Conclusions: 3BNC117 delays viral rebound in participants sensitive to 3BNC117. We detected significant changes in reservoir structure over the 25 week interval between leukaphareses, but these changes did not appear to be mediated by 3BNC117. Isolated latent viruses were highly similar but generally not identical to rebound viruses.

OP 6.6

Single romidepsin infusions do not increase HIV expression in persons on ART (A5315)

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Background: We sought to determine the lowest, safest, effective dose of romidepsin(RMD)for induction of HIV expression.

Methods: Three single-dose cohorts (0.5 mg/m^2, 2 mg/m^2, 5 mg/m^2) of HIV-infected participants were enrolled in a double-blind, randomized, placebo-controlled (3:1 active/placebo; target 15/cohort) study. Enrollees were virally suppressed on EFV, RAL, or DTG-containing ART with plasma HIV RNA ≥0.4 but <50 cps/mL. Viremia was measured by single copy assay (SCA) before and after RMD/placebo 4 hr infusion at hrs 6, 12, 24, 48, days 7, 14, 28.Cell-associated HIV DNA and unspliced RNA were measured by qPCR in resting CD4+ cells pre- and post-infusion (hr 24; day 14). Histone-3

acetylation (H3-Ac) was measured by flow in total CD3+ T-cells pre-infusion; hrs 12, 24, 48; days 7, 14, 28. RMD was measured pre- and post-infusion; hrs 4, 6, 12, 24. Pre-specified primary comparisons were between the pooled RMD and pooled placebo groups using the Wilcoxon test.

Results: 43 enrolled (36 RMD; 7 placebo). All completed the infusions.Median RMD levels at hr 4 were 12.0, 75.2, 89.0 ng/mL in the 0.5, 2.5 and 5 mg/m^2 cohorts. The primary efficacy measure of SCA change from pre-infusion to the average of 24 and 48 hr post was similar between the pooled RMD and placebos (median: 0.12 vs. 0.12 log10 cps/mL, p=0.88, [95% CI on difference: –0.48, 0.33]). No significant increases in any virologic measure or in H3-Ac were observed (p > 0.05).

Conclusions: Single RMD doses were well-tolerated but did not increase HIV expression OR H3-Ac.

PP 6.0

Higher rectal p24 levels correlate with poor CD4 recovery in treated HIV infection

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Background: Gut tissue harbors high levels of HIV DNA and RNA during antiretroviral therapy (ART)-mediated viral suppression, but less is known about HIV protein expression in this compartment, which is required for immune-based clearance strategies and may be more likely than total HIV transcripts to impact systemic immune activation and CD4+ T cell recovery.

Methods: HIV gag p24 protein was measuredusing an ultrasensitive digital ELISA in CD4+ T cells isolated from cryopreserved rectal tissue biopsies (and PBMC in those with samples) from viremic (n=10 with plasma HIV RNA level >4 log10 copies/ml), ART-suppressed (n=7 immunologic non-responders [INR] with CD4+ T cell counts <350 and n=7 immunologic responders [IR] with CD4+ T cell counts>500 cells/mm3) and HIV-uninfected participants (n=5) in the SCOPE cohort.

Results: HIV gag p24 protein levels in rectal and peripheral blood (PB) CD4+ T cells were moderately correlated among all participants (r: 0.54, P=0.0169); however, when restricted to ART-suppressed participants, there was no evidence for a correlation between PB and rectal p24 levels (P=0.35).Rectal p24 levels discriminated between viremic, ART-suppressed, and HIV-uninfected participants much better than PB p24 levels (see Figure).While there was no difference in PB p24 levels, ART-suppressed INR had significantly higher median rectal p24 levels than IR (0.024 vs. 0.009 per million rectal CD4+ T cells, P=0.009).Among all ART-suppressed participants, higher rectal p24 levels were associated with lower CD4 counts (r: –0.69, P=0.006).

Conclusions: Greater rectal HIV gag p24 protein expression is strongly associated with poor CD4+ T cell recovery in PB during ART-mediated viral suppression and may not be accurately reflected by PB p24 expression. These findings suggest a potential impact of gut HIV protein expression on immune recovery during ART and highlight the need to assess HIV protein expression in gut tissue (as opposed to simply PB) in studies of immune-based clearance interventions.

PP 6.1

The CCR5-agonist maraviroc reverses HIV latency, results from *ex vivo* studies and a randomized placebo controlled clinical trial

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Background: One strategy to eliminate latently infected cells in HIV-infected individuals on antiretroviral therapy (ART) is to activate these cells with latency reversing agents (LRAs). The clinically approved CCR5-agonist maraviroc (MVC) has been shown to increase HIV transcription in vitro and may therefore be an LRA. We investigated the effect of MVC on T-cell activation, HIV production in vitro, ex vivo and in vivo during a MVC-intensification trial.

Methods: Immune non-responders participated in a double-blind, placebo-controlled trial of MVC intensification. Using ddPCR, we assessed changes over 8 weeks in total HIV-DNA, 2-LTR circles, HIV-RNA expression and mRNA expression of TNF-a, IFN-y, IL-10 and IL-6. Plasma levels of CCR5 ligands and immune activation markers were analyzed. We assessed the effect of MVC on cell-associated unspliced HIV-RNA in CD4+ T-cells from HIV-1 infected individuals on ART. Impact of MVC on NF-κB phosphorylation was investigated by Western Blot.

Results: Patient characteristics, immunological and virological baseline values did not differ between the MVC-intensification and placebo group. A significant difference in relative cell-associated unspliced HIV-RNA was detected (MVC increase 1.7 fold (n=10); placebo decrease 4.2 fold (n=5); p=0.03). We also measured a 2.3 fold increase in plasma CCR5 ligand MIP-1β in the MVC group. Additionally, a significant difference in NF-kB regulated gene expression was observed; increase in the MVC group and decrease in placebo (IFN-y p=0.02; IL-6 p=0.03). No differences in total HIV-DNA, 2-LTR circles and plasma activation markers were observed. Ex vivo, in CD4+ T-cells from HIV infected patients treated with 1μM MVC relative to DMSO we observed a 2.7 fold increase in unspliced cell-associated HIV-RNA (n=4). In vitro, a 2.5 fold increase in phosphorylated NF-κB was observed in uninfected total CD4+ T-cells treated with 1μM MVC.

Conclusions: In HIV-infected individuals on suppressive ART, the addition of MVC led to an increase in CCR5-ligand expression, NF-κB regulated gene expression and HIV-RNA transcription. These data are in line with our ex vivo and in vitro observation of MVC induced HIV transcription and phosphorylation of NF-κB. Together, these data indicate that MVC has a dual role as an antiretroviral agent and as a latency reversal agent.

PP 6.2

Immunocapture identification of myeloid cell-derived HIV in CSF that is evolutionarily divergent from plasma virus

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Background: Accumulating evidence that myeloid lineage cells harbor HIV was further supported by the finding of viral rebound from macrophage after treatment interruption in humanized mice devoid of all T lymphocytes.Given that myeloid cells are the predominant immune population in the central nervous system (CNS), and some possess half-lives of months or years, infection of these cells could serve as persistent reservoirs of HIV.

Methods: We examined plasma and cerebrospinal fluid (CSF) of 10 treatment-experienced persons using an HIV particle immunocapture algorithm, targeting cell surface proteins that embed in virion envelopes during budding, to segregate T lymphocyte-derived virus particles from those expressed from myeloid cells.The performance of the capture method was validated on HIV cultured from sorted

immune cells.We surveyed for HIV genomic variants as evidence of distinct HIV reservoirs. CSF HIV RNA expression was compared to provirus detection in CSF cells.

Results: The majority of reverse transcriptase bulk sequences from plasma virus specimens were highly similar to genotypes of CSF HIV.However, an individual with long-term infection (30 years) had a $CD14^+$ HIV variant in CSF that was highly divergent from the virus currently expressed in plasma prior to re-initiating treatment.This CSF variant had drug resistance mutations (K103N/M184V/Y188L) not detected in any of the plasma fractions from the same time.The person with CD14-associated drug resistance was one who maintained low-level proviral DNA (27 copies/10^6 cells) in CSF cell pellets after subsequent treatment suppression of viral RNA.Additionally, a $CD3^+$ variant in the CSF of another individual exhibited nucleotide polymorphisms that differed from plasma sequences.

Conclusions: Rare instances of detectable cell type-specific variants revealed that HIV can be maintained within different immune cell lineages.The finding of highly divergent $CD14^+$ virions, indicative of CSF macrophage or CD14+ dendritic cell sources, in a long-term infection suggests that variants persist in myeloid cells.Moreover, $CD3^+$ virion polymorphisms unique to CSF suggested sequestered CNS lymphocyte pools that are subject to selective pressures apart from the blood compartment.

PP 6.3

Safety and potential impact of auranofin on the viral reservoir in HIV positive individuals under mega-ART

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Background: Residual inflammation-related proliferation of HIV reservoirs prompted suggesting antirheumatic drugs for treatment of HIV/AIDS. We present evidence supporting the use of the gold salt auranofin in HIV+ individuals under ART [www.clinicaltrials.gov; ID: NCT02961829].

Methods: We analyzed the data from four of the six arms of the NCT02961829 clinical trial with 5 patients each (all male) with: (NCT02961829's Group 1) no intervention, i.e. continuation of ART (tenofovir/3TC and efavirenz), (NCT02961829's Group 2) mega-ART (ART + dolutegravir and maraviroc), (NCT02961829's Group 4) mega-ART plus auranofin, (NCT02961829's Group 5) intensified ART (ART + dolutegravir). Total and integrated viral DNA was measured by qPCR techniques following in-house analyses aimed at ruling out the effect of PCR inhibitors.

Results: Auranofin treatment was well tolerated. No major side effects were reported. In line with the antiproliferative effect of the drug, the auranofin-treated group showed transient decreases in CD4 counts at weeks 8 and 12, which were significant to repeated-measures ANOVA ($P = 0.0031$) but not pronounced enough to reach statistical significance in the Turkey post-test. Auranofin decreased viral DNA in PBMCs as compared to ARV-only containing regimens at week 20 ($P = 0.036$). We then compared the viral reservoir dynamics from Groups 2 (mega-ART) and 4 (mega-ART + auranofin) by measuring the integrated viral DNA by alu-PCR. The method of Tan et al. showed a significant decrease of the integrated DNA over time in both the mega-ART-only group and in the mega-ART/auranofin group at week 20 ($P = 0.044$; two-way ANOVA), with a trend toward a steeper decrease in the mega-ART/auranofin group ($P = 0.058$). The method of Brussel et al. instead showed a significant increase in integrated DNA in the mega-ART-only group ($P = 0.030$) but not in the auranofin-treated group.

Conclusions: The data allow concluding conclude that auranofin is well tolerated and suggest an inhibitory effect on the viral reservoir dynamics during mega-ART treatment, which should be tested in future clinical trials with larger numbers of patients. Discordance among methods for viral reservoir detection however remains a major problem affecting AIDS cure research at present.

PP 6.4

Balancing risk-benefit ratio in donors of gut biopsy samples for HIV persistence research

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Background: Despite of effective antiretroviral therapy (ART), HIV reservoirs persist in long-lived memory CD4 T-cells present in the blood and in tissues impeding cure. Gastro-intestinal (GI) tract has been shown contain the vast majority of HIV reservoirs in the body. Therefore, interest grows to obtain GI tissue samples for HIV persistence studies posing an ethical challenge to provide study volunteers adequate information on risks and benefits. In this study, we determined the risks and benefits of undergoing gut biopsy procedures for HIV pathogenesis and reservoir studies.

Methods: Based on a group discussion with physicians and community representatives, colonoscopy was chosen over flexible sigmoidoscopy to obtain GI biopsies in persons aged 50+ years. From March 2015 to May 2017, 30 ART-treated and nine HIV-uninfected participants were recruited. Thirty gut mucosal biopsies were collected using colonoscopy. Pre-cancerous polyps were removed when present and abnormal mucosal findings were biopsied for pathological analysis. Participants were followed-up to assess potential discomfort subsequent to colonoscopy.

Results: A similar age and gender distribution was observed in HIV-infected vs. uninfected group with more Men who have sex with men (MSM) in the former group. Abnormal colonoscopic findings were observed in 43.6% of all the participants and did not differ by HIV status. A total of 24 polyps were removed with a higher mean number of polyps removed in HIV-infected vs. uninfected participants (1.7 vs 1.0; p=0.013). The number of polyps marginally correlated with CD4/CD8 T-cell ratio. Based on our findings, colonoscopic examination was safe to use for gut biopsy procedures where almost half of the participants had polyps removed.

Conclusions: Colon cancer screening is an ancillary benefit to the participants in HIV cure research and mitigates the burdens of invasive procedures. Dialogue between community representatives and clinical researchers can increase participation and advance HIV cure research.

PP 6.5

Determinants of early ART initiation during primary HIV infection: implications for HIV cure research

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Background: ART does not cure HIV due to the existence of HIV reservoirs in long-lived cells. Early initiation of ART has been associated with a smaller reservoir size. Herein, we investigated the time trends and factors associated with early ART Initiation in the Montreal Primary HIV Infection (PHI) Study.

Methods: The PHI Study is a prospective cohort established in three community medical centres (CMCs) and two university medical centres (UMCs). Recently diagnosed adults treated within 180 days of baseline visit (Early ART group) were compared to late ART group on their socio-demographic, behavioral and laboratory parameters.

Results: Among participants recruited from 1996 to 2015; 348 had a documented PHI and long-term follow up. The median (IQR) age of participants was 35 (28; 42) years and the majority were male (96%), having paid employment (63%), men who have sex with men (MSM) (78%) and 1–4 sexual partners in the last three months (70%). Participants presented with a median (IQR) HIV plasma viral load of 4.6 (3.7; 5.3) log10 copies/mL, CD4 count of 510 (387; 660) cells/µL and were recruited in community medical centres (52%) or university medical centres (48%). Early ART initiation was observed in 47% of the participants and the trend followed a V-shaped curve with peaks in 1996–97 (89%) and 2013–15 (88%) with a dip in 2007–09 (22%). Multivariate analyses showed that having paid employment (aOR: 2.43; 95% CI: 1.19, 4.95), lower CD4 count (aOR per 50 cell increase: 0.93; 95% CI: 0.87, 0.99) and care at university medical centres (aOR: 2.03; 95% CI: 1.06–3.90) were independently associated with early ART initiation.

Conclusions: In the context of universal access to care, early ART initiation was associated with higher socio-economic determinants. Montreal PHI Study represents an important source of candidates with low HIV reservoirs to participate in cure research.

PP 6.6

Lymph node CA-DNA strongly correlates with CD4+Tc count, plasma viral load and CD4/CD8 ratio during chronic HIV infection

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Background: Lymph nodes (LN) are a main anatomical site for HIV replication and persistence. However, the impact of LN proviral reservoir size on peripheral blood (PB) prognostic markers of clinical disease has not been addressed. We assessed the relationship between LN and PBMC cell associated HIV DNA (CA-DNA) with clinical prognostic markers (plasma viral load [pVL], CD4 T cell count and CD4/CD8 ratio) in viremic and ART-suppressed individuals.

Methods: Paired cervical LN and PBMCs were obtained from 25 viremic and 6 ART-suppressed individuals. LN and PBMC CA-DNA were measured by qPCR and normalized to copies/million cells (cp). Statistical analysis were performed with GraphPad Prism using nonparametric tests.

Results: During untreated HIV infection, a significant correlation was detected between the levels of LN and PBMC CA-DNA (Spearman r=0.88, P<0.0001). However, levels of CA-DNA were 4 times higher in LN compared to PBMCs (median 2022 vs. 464 cp, P <0.0001). In viremic individuals, LN CA-DNA strongly correlated with all prognostic markers accessed: pVL (r= 0.64, P=0.0006); CD4+cell count (r=–0.47, P=0.02); CD4% (r=–0.56, P=0.004) and CD4/CD8 ratio (r=–0.50, P=0.01). The association between these clinical markers and PBMC CA-DNA were far weaker: pVL(log) (r= 0.61, P=0.002); CD4+ cell count (ns); CD4% (r=–0.43, P=0.04) and CD4/CD8 ratio (ns). ART treated individuals harbored lower CA-DNA levels in LNs (viremic vs ART-suppressed: median 2022 vs. 315 cp, P=0.0007) and PBMCs (median 464 vs. 233 cp).

Conclusions: During untreated infection, the establishment of CA-DNA in LN strongly correlated with clinical prognostic markers, such as pVL, CD4+ cell count, and CD4/CD8 ratio.The stronger effect of LN vs. PBMC CA-DNA support the importance of the tissue HIV reservoir on clinical disease progression. Further studies are needed to assess the trafficking of virus and infected cells within the tissue and peripheral compartments.

PP 6.7

Rapid antiretroviral therapy of blood donors with acute and recent HIV infection: a preliminary report from the Monitoring and Acute Treatment of HIV Study (MATHS)

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Background: All blood donations in South Africa are tested in parallel for HIV antibody and RNA using individual-donation nucleic acid testing (ID-NAT), allowing detection of acute (RNA+/ ab-; Fiebig stages I and II) and recent (RNA+/ab+; Fiebig stages III to VI) HIV infections. We will investigate whether more rapid initiation of antiretroviral therapy (ART) correlates with smaller HIV reservoir.

Methods: A prospective cohort study enrolls 50 Acute (Fiebig I/II) and 50 Recent (Fiebig III-VI) HIV infected blood donors. HIV antibody (Abbott Prism) and HIV RNA ID-NAT (Grifols) are measured on all blood donations and recency (< 130 days) is detected by a limiting antigen (LAg) avidity assay (Sedia). Eligible donors are consented and rapidly treated with RAL/TDF/FTC X 6 months followed by EFV/TDF/FTC. Plasma RNA and cell-associated RNA and DNA are measured by ultrasensitive TMA (Hologic) and real-time PCR.

Results: From October 2015 to September 2017 we enrolled 46 (42 evaluable) donors with Acute HIV and 25 (24 evaluable) donors with Recent HIV. The Acute group had median age 29 years, 29 females, majority Black, median HIV RNA 319,835 copies/mL and mean CD4 453 cells/mm3. Enrollment occurred 15 days after donation and ART was initiated 2 days after enrolment (medians). Of 32 Acutes who were Fiebig stage I/II at blood donation 13 (40%) were still Fiebig I/II at enrollment. Viral suppression (<20 copies/mL) occurred after a median of 38.5 days on ART. Mean cell-associated RNA was 10^4 copies per 10^6 PBMC at enrollment, falling to 10^1–10^2 copies per 10^6 PBMC after 6–8 weeks on ART, with no difference by index Fiebig stage. Mean cell-associated DNA was 10^2 copies per 10^6 PBMC at enrollment, falling to 0–50 copies per 10^6 PBMC perhaps more slowly in Fiebig I/II than Fiebig III-VI subjects.

Conclusions: This study provides proof of principle that a partnership between a national blood service and an HIV treatment unit can detect and rapidly treat persons with Acute and Recent HIV infection. Initial laboratory results suggest that both plasma viremia and HIV reservoir are reduced soon after ART initiation, with perhaps little difference by Fiebig stage at enrollment.

PP 6.8

Effect of switching to integrase inhibitor on the HIV reservoir in ileum biopsies

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Background: Antiretroviral therapy switching towards newer drugs is a potential strategy to impact the HIV reservoir. The effect of integrase inhibitors on the HIV reservoir in tissues remains unknown. Thus, we evaluated the effect of switching from PI to dolutegravir (DTG) in cART on the HIV reservoir size in blood and ileum biopsies.

Methods: INDOOR study, a phase-IV and opened clinical trial, randomly included 44 HIV-1 infected subjects on effective cART: 22 switched from PI to DTG based-cART (switch group), and 22 remained in PI-based regimens (control group). We collected 4 to 8 endoscopic ileum biopsies and blood samples at weeks 0 and 24 from 33 subjects: 13 from the switch group and 20 from the control group. Total HIV DNA was determined by ddPCR in peripheral blood mononuclear cells (PBMC) and in sorted CD45+ cells from ileum biopsies.

Results: This switching strategy was safe and well tolerated for all the patients, with no changes in viremia suppression(<50 cp/ml). Two subjects, one from each arm, did not finish the study. One with prior psychiatric history suffered a psychotic attack and the second was lost to follow-up at the last visit; however, his last viral load was undetectable. We detected total HIV DNA in all samples, and a significant correlation of the HIV reservoir size was observed between tissue and blood samples (p=0.01, Rho=0.43 at week 0). Moreover, the reservoir size was consistently higher in tissue CD45+ cells than in PBMC in both groups (p<0.01). However, we did not observe significant longitudinal changes in the total HIV reservoir size, either in CD45+ cells of ileum biopsies or in PBMC, in any study group.

Conclusions: INDOOR study evaluated for the first time changes in the HIV reservoir size in ileum biopsies in individuals switched from PI- to DTG-based cART. This switching was safe and well tolerated, but had no impact on the HIV reservoir size in CD45+ cells of ileum.

PP 6.9

Quantification of undetectable plasma HIV RNA

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Background: Plasma HIV RNA or viral load (VL) is measured in clinical practice and trials of antiretrovirals using FDA-cleared assays such as COBAS TaqMan HIV-1 Assay v2.0. The TaqMan assay provides quantification of viremia at or above 20 copies/mL, but lower values are reported as "<20" or "Target Not Detected" (TND). Current "kick & kill" HIV eradication strategies may require more sensitive assays to detect changes in low-level persistent viremia. Here, the novel integrase single-copy assay (iSCA) (Cillo JClinMicro 2013) was evaluated for measurement of low-level persistent viremia in a large number of clinical trial samples.

Methods: Plasma samples were from Week 24 visits of a Phase 2 study in previously treatment naïve HIV-1-infected patients treated with a single tablet regimen containing an integrase inhibitor. HIV-1 RNA levels were assessed at a central laboratory using HIV-1 TaqMan 2.0 Assay (Roche Diagnostics, Indianapolis, IN). The iSCA assay was performed in a blinded fashion on matched samples (University of Pittsburgh) and results from the 2 assays were compared.

Results: Paired TaqMan/iSCA data were obtained for 151 HIV-infected adults. All participants were on treatment and virologically suppressed (mean 110 days) at week 24. Most samples (117/151, 77%) had non-quantifiable TaqMan result, either <20 copies/mL (n=44) or TND (n=73). Quantification was achieved with iSCA for all 117 samples (mean VL 2.6 copies/mL for 73 samples with TND; mean VL 8.2 copies/mL for 44 samples with <20 copies/mL). Zero copy control samples included with each assay run were all negative for HIV RNA (<1 copy).For samples quantified with both assays (n=34), iSCA values were slightly lower than TaqMan (mean VL of 29.5 copies/mL compared to 61.4 copies/mL, respectively).

Conclusions: In this large sample collection from virologically suppressed HIV-infected adults, use of iSCA led toquantification of low-level viremia below the limit of detection of the TaqMan assay in 77% (117/151) of previously non-quantifiable plasma samples. This dataset emphasizes the value of the iSCA over classical HIV VL assays for measurement of low-level viremia and its potential for use in HIV cure studies to assess whether experimental interventions alter viremia.

PP 6.10

The critical importance of social sciences in early-phase HIV cure research: what's in it for biomedical HIV cure scientists?

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Background: Social sciences can guide meaningful stakeholder engagement, ensure the ethical conduct of research, and enable the inclusion of key essential populations in research. In the HIV cure research field, social sciences and ethics research are lagging behind biomedical research.

Methods: We conducted an extensive survey (n = 400), key informant interviews (n = 36) and focus groups (n =10) to better understand barriers to HIV cure research participation, perceptions of various HIV cure research strategies, understanding of risks and benefits of HIV cure research, and ethical issues in first-in-human protocols, among people living with HIV and other key stakeholders in the United States.

Results: Perceived clinical risks of HIV cure research are not the only barriers to participation. Factors related to how biomedical HIV cure research teams interact with study participants, as well as psychosocial and mental health support, play a central role in the decision of people living with HIV to participate in early-phase, risky HIV cure research. Research revealed differences in motivations across study types and differences by gender, ethnicity and perceived health status. A lack of consensus remains on what analytical treatment interruptions are ethically permissible. Practical, logistical, tactical, social, ethical, cultural, and economic issues affect the implementation of HIV cure research at all stages of translation. Despite the high willingness to participate in HIV cure research among people living with HIV in the United States, more information is needed about specific risks of HIV cure studies, inclusion/exclusion criteria, and specific patient populations that could qualify for trials.

Conclusions: Social sciences are a critical adjunct to ongoing biomedical HIV cure research efforts. Social sciences can contribute considerably to HIV cure research, by assessing community knowledge, characterizing perceptions of specific HIV cure modalities, examining language effective for recruitment and engagement, evaluating clinical trial design and product acceptability, identifying factors affecting retention in research, avoiding community opposition, proactively recognizing ethical issues, and ensuring a patient-centered HIV cure research agenda. Failure to incorporate social sciences could lead to HIV cure strategies that are unacceptable to people living with HIV and their care providers.

PP 6.11

Stimulating cellular locomotion using α1PI therapy to eradicate reservoirs without adverse effects

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Background: Whereas α1proteinase inhibitor (α1PI, α1antitrypsin) was thought to act primarily as a protease inhibitor, it is now known to primarily act in lymphocyte locomotion. Active α1PI complexed with cell-surface human leukocyte elastase (HLECS) stimulates polarization of functionally-related receptors including chemokine receptors and CD4 at the leading edge of a migrating lymphocyte. The α1PI-HLECS complexes subsequently bind to members of the LDL receptor family inducing internalization of the receptor aggregate at the trailing edge of the cell. Internalization releases the cell from tissue allowing forward locomotion and uptake of nutrients and viruses including CD4-bound HIV-1. At sites of infection or inflammation,

α1PI is inactivated by proteases other than HLECS and locomotion halts precisely where the pathogenic insult exists. In HIV-1 disease, Diffuse Infiltrative Lymphocytosis Syndrome is a complication in which lymphocytes infiltrate glands, lungs, nervous system, kidneys, liver, and digestive tract. The lymphocyte infiltrates are phenotypically comparable to circulating lymphocytes and are surrounded by monocytic cells that express HIV-1 proteins. This scenario suggests that α1PI is inactivated within these sites thereby allowing infected lymphocytes to travel into tissue, yet preventing them from traveling out or infecting new cells.

Methods: HIV-1-infected subjects (NCT01370018, n=4) received 8 weekly infusions of α1PI (120mg/kg, Zemaira, CSL Behring). One subject opted to remain in the study for 12 weekly infusions. Inclusion criteria were: i) active α1PI below 11mM; ii) one year history with CD4+ lymphocytes at 150–300 cells/ml; iii) absence of HIV-1 disease progression; iv) adequate suppression of virus (<50 HIV RNA/ml); and v) history of compliance with antiretroviral medication. Each subject was infused at the same time of day and same day of week.

Results: Following initiation of α1PI treatment, serial blips of HIV-1 appeared in all HIV-1 patients (n=4) despite maintenance on antiretroviral therapy. The blips resolved in the following week of α1PI treatment suggesting that the emerging infected cells were rapidly cleared.

Conclusions: Using α1PI treatment, eradication of HIV-1 reservoirs throughout the body may be accomplished. We are developing an orally-available molecule that acts as an α1PI surrogate that is effective and safe in mice.

Session 7: New therapeutic approaches I

OP 7.0

Early lessons from shock and kill trials

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Pharmacologically induced expression of latent virus is investigated as part of a cure for HIV-1 infection. Recent data from clinical trials show that short-term administration of a latency-reversing agent (LRA) may increase HIV-1 transcription, HIV-1 protein expression and plasma HIV-1 RNA. So far, reversal of HIV-1 latency by histone deacetylase inhibitors and other LRAs has not been associated with a reduction in the size of the latent reservoir. Possible explanations for the lack of an effect on the size of the latent HIV-1 reservoir include insufficient immune response against virus-expressing cells, the presence of cytotoxic T lymphocyte (CTL) escape variants, and/or an insufficient degree of latency reversal achieved with current approaches. Importantly, these early studies of LRAs were primarily designed to investigate their ability to perturb the state of HIV-1 latency. Newer studies have attempted to either combine LRAs with interventions such as therapeutic HIV-1 vaccines aimed at improving the killing of reactivated cells, or to test compounds such as TLR7 and TLR9 agonists which may have dual effects as both LRAs and enhancers of antiviral immunity. This talk will focus on the lessons we have learned from these early 'shock and kill' trials and review recent published as well as unpublished data.

OP 7.1

Improved HIV-1 clearance with BIT225 in the HIV-1 infected humanised mouse model

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Background: Recent studies in the humanized mouse model have confirmed the persistence of HIV-1 replication in tissue macrophages during treatment and that these infected cells act as a viral reservoir.BIT225 is a first in class antiviral drug that blocks Vpu ion channel activity resulting in disrupted HIV-1 assembly within the host cell and a substantial loss of infectivity of the progeny virus. BIT225 demonstrates good anti-HIV-1 activity in vitro and in the clinic with preferential antiviral activity in cells of the myeloid lineage.

Methods: NOD/Shi-scid/IL-2Rγnull immunodeficient mouse strain (NOG) were humanized with CD34+ hematopoietic stem cells, enhanced with a cocktail of human cytokines in order to increase monocyte number and infected with HIV-1 (Yu2 strain) to high plasma viral load (pVL) levels.Two groups (n=8/group) were matched for monocyte number and HIV-1 burden prior to treatment with combination antiretroviral therapy (cART: RAL/3TC/TDF) ±BIT225 at 2 mg/mouse/day for 5 weeks.Once the mice reached undetectable HIV-1 pVL's, an analytical treatment interruption (ATI) with ±BIT225 only, was conducted to monitor HIV-1 pVL rebound.

Results: HIV-1 pVL decreased rapidly from week 0 to week 5 in both groups. The addition of BIT225 increased the initial rate of HIV-1 clearance, with a significant effect on the pVL decay slope between days 0 and 10 when compared to the placebo group (p=0.03). After initiation of an ATI, pVL increased in both groups. However, HIV-1 rebound was slower in the BIT225 treated group and unlike the placebo group, did not return to pre-treatment levels at week 10 (p=0.01).

Conclusions: The faster pVL clearance rates with BIT225 are likely a result of the additional targeting of the infected myeloid compartment; cells that cART alone did not impact on.Faster viral clearance may reduce the size of the viral reservoir as suggested by the ATI, as rebound was slower with BIT225 treatment.A longer BIT225 treatment period of the mice, with undetectable pVL's, could potentially eradicate this macrophage reservoir.

OP 7.2

In vivo suppression of HIV rebound by didehydro-Cortistatin A, a 'block-and-lock' strategy for HIV-1 cure

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Background: HIV-1 Tat activates viral transcription and limited Tat-transactivation correlates with latency establishment. We postulated a 'block-and-lock' functional cure approach based on properties of the Tat-inhibitor didehydro-Cortistatin A (dCA).

Methods: The 'block-and-lock' hypothesis was investigated in human CD4+T cells isolated from aviremic individuals and in the bone marrow-liver-thymus (BLT) mouse model of HIV latency and persistence. Using the OM10.1 cell line model of HIV-1 latency, we investigated the epigenetic signature of the HIV-1 promoter after long term treatment with dCA by MNase nucleosomal protection assays, and ChIP.

Results: Utilizing the human CD4+T cells isolated from aviremic individuals, we showed that dCA with ART accelerated HIV-1 suppression and prevented viral rebound after 25 day treatment interruption, even during strong cellular activation.

Rearrangement of the nucleosomes and loss of protection from Nucleosome-1 regions is observed upon HIV-1 reactivation from latency. In OM10.1 cells treated with dCA we observed higher Nuc-1 occupancy, with only small changes upon stimulation with latency reversing agents. This result was consistent with a drastic inhibition of RNAPII recruitment to the HIV promoter and genome.

In the BLT mouse model, adding dCA to ART suppressed mice systemically reduced viral mRNA in tissues, and significantly delayed and reduced viral rebound levels upon treatment interruption.

Conclusions: We demonstrated in the setting of full HIV suppression, that dCA 1) reduces cell-associated viral RNA systemically, 2) significantly delays viral rebound upon treatment interruption and 3) reduces viral rebound levels by several orders of magnitude. These results strongly support the rational for the inclusion of specific HIV transcriptional inhibitors with current therapies for 'block-and-Lock' cure strategies.

OP 7.3

Properties of eCD4-Ig relevant to reducing the viral reservior

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Background: eCD4-Ig is an exceptionally broad HIV-1 entry inhibitor that neutralizes, with IC_{80}s<10 µg/ml, all270 HIV-1, HIV-2 and SIV isolates it has been tested against.eCD4-Ig's breadth and potency derives from the fact that it closely and simultaneously mimics the HIV-1 receptor CD4 and the HIV-1 coreceptor.eCD4-Ig can be used in an AAV-expressed vaccine alternative or functional cure, and as part of a 'kick-and-kill' strategy to reduce the scale of the viral reservoir.

Methods: We have studied the ability of AAV-expressed eCD4-Ig to protect rhesus macaques from SHIV-AD8 and SIVmac239.We have compared the ability of HIV-1 to escape eCD4-Ig and broadly neutralizing antibodies.We have developed new more stable and

bioavailable forms of eCD4-Ig, and we have investigated the ability of eCD4-Ig to mediate ADCC alone and in concert with sera of HIV-1-positive individuals.

Results: As a vaccine alternative, we have shown that AAV-expressed eCD4-Ig can protect rhesus macaques from repeated high-dose viral challenges with two divergent and difficult-to-neutralize HIV-1-like viruses, SHIV-AD8 and SIVmac239.These doses are greater than what most humans will ever encounter.As a component of a 'kick and kill' strategy, we have shown, eCD4-Ig is much harder to escape than broadly neutralizing antibodies, suggesting that it can recognized the vast majority of reactivated virus.Moreover, eCD4-Ig, uniquely among agents that are efficient at both neutralization and ADCC, markedly improves the endogenous ADCC activity of patient sera.It does so by altering the conformation of HIV-1 Env, allowing V3 and CD4i antibodies to bind Env.

Conclusions: In short, eCD4-Ig has key advantages in a kick-and-kill cure strategy: it is 100% broad and more difficult to escape than antibodies, its breadth and potency are established in vivo, it mediates very efficient ADCC activity, and it enlists otherwise dormant antibodies in patient sera to further mediate ADCC.

OP 7.4

Treatment with native heterodimeric IL-15 increases cytotoxic lymphocytes in lymph nodes and reduces SHIV RNA

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Background: Heterodimeric interleukin-15 (hetIL-15) activates and expands cytotoxic T and NK cells, which suggests that the cytokine could be useful for the treatment of malignancies and HIV infection. Based on these properties, hetIL-15 is currently evaluated in humans for the treatment of cancer. We study the effects of hetIL-15 in infected macaques to evaluate its use in HIV infection.

Methods: Rhesus macaques, either chronically infected by SHIV or uninfected received injections of hetIL-15 over 2 weeks using increasing doses of cytokine (step-dosing). At the end of the treatment, the animals were sacrificed and the hetIL-15 effects on different lymphocyte populations isolated from tissues collected at necropsy were monitored by multi-parametric flow cytometry and quantitative multiplexed confocal microscopy (histo-cytometry). Cell-associated viral RNA and plasma viral load was measured by quantitative PCR.

Results: This protocol was safe in rhesus macaques and resulted in systemic expansion (Ki67+) of CD8+ T lymphocytes and NK cells with higher granzyme B content. These expanded cell populations were found in both effector sites, such as liver, vagina and rectum, and secondary lymphoid tissues. Importantly, a significant increase in cytotoxic effector memory CD8+ T cells was found in lymph nodes (LN) from all hetIL-15-treated macaques. CM9 tetramer staining demonstrated that the increase of CD8+ effector T cells in lymphoid organs included actively proliferating SIV-specific T cells with higher granzyme content. Imaging analysis by histo-cytometry revealed that these effector CD8+ T cells infiltrated the B cell follicles where chronically infected follicular helper CD4+ T cells are located.Following hetIL-15 treatment, cell-associated RNA was decreased in LN and plasma viral load was also decreased.

Conclusions: Step-dose administration of hetIL-15 is a well-tolerated regimen that results in systemic activation and expansion of cytotoxic leukocytes that infiltrate areas where chronic HIV-infected cells reside. These results suggest that hetIL-15 could be useful in disrupting sanctuary sites within the B cell follicles and reducing long-term viral reservoirs in HIV-1 infected individuals, thus contributing to a functional cure of the infection.

OP 7.5

The human IL-15 superagonist complex ALT-803 drives SIV-specific CD8+ T cells into B cell follicles

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Background: There is an urgent need for alternate approaches to activate and clear the HIV reservoir that do not negatively impact immune function. An added hurdle to clearing the reservoir is the exclusion of CD8+ T cells from B cell follicles, anatomical sites that harbor 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, ALT-803, as an immunostimulatory molecule in chronically SIV-infected rhesus macaques.

Methods: SIV-infected rhesus macaques were treated with a single intravenous dose of 100 ug/kg ALT-803 and were subsequently assessed for intrafollicular migration of SIV-specific CD8+ T cells via in situ immunofluorescence staining of lymph nodes with MHC-class-I tetramers. Additionally, RNA in situ hybridization was employed to determine the number of SIV-producing cells within the follicles and extrafollicular regions of the lymph node.

Results: ALT-803 activated NK cells and memory T cells, causing them to undergo proliferation and home to secondary lymphoid tissues, an anatomical location of the viral reservoir. In situ MHC-class-I tetramer staining confirmed increased numbers of SIV-specific CD8+ T cells in lymph node, and revealed that the effector cells trafficked into B cell follicles. Accordingly, lower numbers of SIV-producing cells were found within B cell follicles in elite controllers post ALT-803 treatment indicating immune mediated clearance.

Conclusions: IL-15 superagonist, ALT-803, triggers massive proliferation of NK cells and CD8+ T cells and also reactivates quiescent SIV. ALT-803 also drives activated NK and CD8+ T cells into lymph nodes, and allows for SIV-specific CD8+ T cells to enter B-cell follicles harboring latently-infected CD4+ TFH cells. The ability of ALT-803 to potentially mediate the 'shock' and 'kill' and to grant CD8+ T cells access to lymph node sanctuary sites makes it an appealing candidate for studies aimed at durable cART-free HIV remission.

OP 7.6

Preclinical development of a bispecific HIV x CD3 DART molecule that redirects T cells to kill HIV envelope (env)-expressing cells

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Background: MGD014 is a bispecific DART® molecule that targets env-expressing HIV-infected cells for recognition and elimination by CD3-expressing T lymphocytes. The anti-HIV env component is derived from A32, a non-neutralizing mAb broadly reactive against cells infected by diverse HIV-1 isolates. MGD014 contains a human Fc

domain, disabled for Fc-gamma receptor and complement binding, which retains neonatal Fc receptor binding to prolong half-life. Prior proof-of-concept studies have been described: Sung et al. (2015) J Clin Invest 125:4077; Sloan et al. (2015) PLoS Pathog 11:e1005233.

Methods: MGD014 was produced in CHO cells; purified GMP product was 99.6% monomer by size exclusion chromatography.

Results: Surface plasmon resonance demonstrated high affinity binding to CD3(K_D<10 nM) and env proteins (K_D<1 nM). Flow cytometry confirmed MGD014 binding to respective antigens on target and effector cells. MGD014 did not bind HIV-1 virions. MGD014 potently redirected CD8 T cells to kill env-expressing cell lines or CD4 T cells infected by transmitted/founder HIV-1 infectious molecular clones. CD8 T cells from uninfected, normal donors and HIV-infected donors on suppressive ART showed similar potency as effector cells. In ex vivo assays, MGD014 mediated the clearance of rare infected patient resting CD4 T cells following reversal of latency by exposure to clinically relevant doses of vorinostat. When administered to ART-suppressed HIV-infected humanized BLT mice, MGD014 mediated reductions in cell-associated viral RNA in peripheral blood and tissues, and a discernable delay in viral rebound upon ART interruption was observed. In a toxicology study conducted in uninfected, normal cynomolgus monkeys with administration by IV infusion weekly for 6 weeks at 0, 0.1, 1 or 10 mg/kg, MGD014 exhibited linear PK and attained CD3 receptor occupancy levels on T cells of ~2%, 30% or 100% at the low, mid or high dose levels, respectively. There were no MGD014-related toxicities, including no induction of serum cytokines or T-cell activation.

Conclusions: The preclinical data support the clinical development of MGD014 as an immunotherapeutic with potential to facilitate the elimination of virus-infected, env-expressing cells in HIV-infected individuals. A phase 1 study to characterize the safety and tolerability of MGD014 in HIV-infected participants maintained on suppressive ART is in development.

PP 7.0

Therapeutic efficacy of optimized eCD4-Ig proteins in SHIV-infected rhesus macaques

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Background: We recently reported that eCD4-Ig, an entry inhibitor that fuses CD4-Ig to a CCR5-mimetic peptide, is just as potent as, and broader than any broadly neutralizing antibody (bNAb) described to date.We demonstrated that AAV-delivered eCD4-Ig could protect rhesus macaques from multiple escalating doses of SHIV-AD8 and SIVmac239.However, we must first show that the eCD4-Ig protein itself is safe and effective.

Methods: To improve the *in vivo* properties of eCD4-Ig, we made a series of variants by mutating amino acids in CD4 domain 2, the hinge, and CCR5-mimetic peptide.Variants were evaluated for aggregation by dynamic light scattering (DLS).Optimized variants were tested for B and T cell reactivity by flow cytometry.TZM-bl neutralization assays were performed to determine breadth and potency against a panel of 200 clade C isolates.Pharmacokinetics and therapeutic efficacy were assessed in rhesus macaques.

Results: Using DLS, we identified an optimal eCD4-IgG1 and eCD4-IgG2 variant with aggregation temperature above 60°C, similar to those observed with the bNAbs VRC01 (66°C) and PG9 (59°C).Both variants of eCD4-IgG1 and IgG2 had substantially less reactivity against B and T cell lines compared to CD4-Ig.The eCD4-IgG1 and IgG2 variants neutralized all 200 isolates of a clade C panel (median IC50 titers 0.151 µg/mL IgG1, 0.148 µg/mL IgG2).In rhesus macaques, eCD4-IgG1 had a half-life that ranged from 2.77-6.23 days and eCD4-IgG2 had a half-life that ranged from 2.65-7.73 days.Importantly, treatment with either eCD4-Ig variant in SHIV-AD8 infected rhesus macaques decreased viremia by 1.5-2.0 log(10) viral RNA copies/mL.All eight macaques that received a second treatment saw a decrease in viremia, indicating that rebounded virus had not escaped eCD4-Ig.

Conclusions: We conclude that optimized eCD4-Ig proteins could be used to treat HIV-1 infection in infected individuals.It is possible that these optimized eCD4-Ig proteins could be used as a killing agent in a 'shock and kill' cure approach.

PP 7.1

eCD4-Ig promotes ADCC activity of sera from HIV-1-infected patients

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Background: Antiretroviral therapy can provide a functional cure for HIV-1; however, the virus persists in latently infected cells. A sterilizing cure will need to reduce this latent reservoir in order to be fully effective. Antibody-dependent cell-mediated cytotoxity (ADCC) can eliminate HIV-1 infected cells, and may help reduce the reservoir of latent virus in infected patients. Sera of HIV-1 positive individuals include a number of antibodies that recognize epitopes usually occluded on HIV-1 envelope glycoprotein (Env) trimers. We have recently described eCD4-Ig, a potent and exceptionally broad inhibitor of HIV-1 entry that can be used to protect rhesus macaques from multiple high-dose challenges with simian-human immunodeficiency virus AD8 (SHIV-AD8) and that also has potential for use as a therapeutic.

Methods: Using eCD4-Ig in combination with HIV-1 antibodies and HIV-1+ patient sera, we performed ADCC assays using CCR5+ CEM-NKR cells that express luciferase upon HIV infection. In addition, latently infected T cells were stimulated using latency reversing agents (LRAs) and then subjected to ADCC analysis using intracellular p24 staining.

Results: Here we demonstrate that in addition to having potent ADCC activity alone, eCD4-Ig can increase the binding of V3-loop and CD4-induced (CD4i) antibodies to Env. This increase in binding renders HIV-1-infected cells susceptible to ADCC mediated by antibodies of these classes.Moreover, eCD4-IgG2, but not IgG2 forms of the broadly neutralizing antibodies VRC01 and 10-1074, significantly enhances the ADCC activities of antibodies present in serum from infected patients. Additionally, we confirmed the ADCC activity of eCD4-Ig against latently infected cells following in vitro stimulation by LRAs.

Conclusions: The ability of eCD4-Ig to draw out ADCC activity in serum could be useful in a 'shock and kill' approach to a cure by directing antibodies present in infected individuals to kill cells infected with reactivated virus. This study further demonstrates the utility of eCD4-Ig as a therapeutic for treating HIV-1 infected individuals.

PP 7.2

Re-evaluating the peptide repertoire of MHC-E

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Background: A Rhesus CMV SIV vaccine induces a very broad and atypical cytotoxic CD8 T cell response that allows ~50% of vaccinated Rhesus macaques to clear SIV following challenge with SIVmac239. Two thirds of these CD8 responses are restricted by MHC Class II, the remainder by MHC-E. In humans, HLA-E exhibits limited polymorphism, and is expressed on nearly all cell types, including elevated levels on T follicular helper cells – an important reservoir of HIV-1. In contrast to the limited MHC-E peptide repertoire originally described, with methionine at position 2 and leucine at the peptide C terminus, the Mamu-E-restricted SIV epitopes exhibit surprising sequence diversity and no obvious motif. To understand how MHC-E can accommodate these peptides, we are re-examining its peptide repertoire. This should enable us to produce reagents specific for MHC-E presenting selected HIV-1 epitopes for evaluation as therapeutic agents.

Methods: MHC-E is expressed as single chain timers (E-SCT) comprising peptide, β2-microglobulin and heavy chain, and surface expression used as a surrogate for peptide binding affinity. This approach is complemented by conventional in vitro refolding, native protein gel electrophoresis, and crystal structure determination of HLA-E presenting selected epitope peptides. To generate reagents specific for HLA-E presenting particular peptides, HLA transgenic mice have been immunised with refolded peptide-HLA-E and lipid nanoparticle-encapsulated mRNA encoding E-SCTs, and various phage display libraries are being screened.

Results: We show that the peptide repertoires of HLA-E and Mamu-E are very similar. Comprehensive mutation of second amino acid of the presented peptide confirms that amino acids other than the expected methionine and leucine can be accepted in a peptide-dependent manner.We have also refined crystal structures of HLA-E presenting selected epitopes and mutated peptides. Together these data show that HLA-E can bind a broad range of peptides in the conventional manner. However, HLA-E may also fold without added peptide and this form may be receptive to an even broader range of peptides. Which of these peptides are epitopes for HIV specific T cells is now under investigation.

Conclusions: Strategies that can elicit HLA-E restricted anti-HIV-1 T cells may give vaccines that are valuable for prophylactic and therapeutic immunization.

PP 7.3

Designing broad-spectrum gRNAs to target the HIV-1 LTR with CRISPR/cas9-based therapeutic strategies

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Background: HIV-1 persistence during long-term antiretroviral therapy is a major hurdle to a cure. Genomic editing techniques, like the CRISPR/Cas9 system, hold promise to permanently excise the integrated virus genome from an intact host cell. However, due to the rapid mutation rate intrinsic to HIV-1 replication and numerous selective pressures, the virus in patients exists as a collection of distinct genomic variants, termed quasispecies.

Methods: PBMC genomic DNA was isolated from 269 patients in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort as well as from brain and spleen tissue from the National NeuroAIDS Tissue Consortium (NNTC) and the long terminal repeat (LTR) of the HIV-1 quasispecies was sampled using Next Generation Sequencing (NGS). A package of 4 gRNAs was selected based on the training data set, termed the selected molecular gRNA targeting (SMRT) package (SMRT-4P).The efficacy of these SMRT gRNAs was tested in vitro in cell line reporter assays.NGS of the insertion/deletions was also performed.Molecular modeling was used to predict the RNA secondary structures. The SMRT gRNAs were also tested in vitro using a cutting assay to detect a spectrum of viral quasispecies.

Results: The SMRT gRNAs were shown in silico to cleave the entire detectable quasispecies within the test CARES patient Cohort DNA samples and the majority of the NNTC patient samples, in the absence of in silico off-target impact. The package was further predicted to cleave all subtype B North American LTRs from the Los Alamos National Laboratory (LANL) database.The four gRNAs were cloned and transfected into HIV-1 reporter cell lines with subsequent transient expression co-transfection studies demonstrating that the SMRT package effectively decreased reporter gene activity as well as a number of LAI-based molecular clone variants. NGS analysis of the insertions/deletions showed that the TAR secondary structure was altered in many of the cut LTRs.Finally, the SMRT gRNAs showed the ability to cut a large number of patient-derived LTR quasispecies using the in vitro cutting assay.

Conclusions: These studies represent a step towards understanding using excision therapy to target integrated HIV-1 proviral quasispecies.

PP 7.4

In situ multiplex RNA fluorescence imaging of SHIV1157ipd3N4 and anti-HIV CAR T cells to study CAR T cell trafficking to sites of viral reservoir in macaque lymphoid tissues

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Background: Residual HIV gene expression and replication occur even under efficient ART in immunological privileged sites (e.g. B cell follicles, CNS). Chimeric antigen receptor (CAR) T cells have been successfully used in cancer therapy and can potentially serve as a functional cure to limit or eradicate viral reservoirs. CAR T cells retain cytotoxic activity for years, function independently of MHC recognition and can target HIV env-expressing cells. To dissect trafficking of anti-HIV CAR cells to lymphoid tissues and their interaction with infected cells requires advanced in situ imaging techniques. However, CAR-specific antibodies are not available at present, and production is tedious and costly. By contrast, RNA fluorescence in situ hybridization-based imaging techniques can be adopted to various CAR constructs and virus strains within weeks.

Methods: To study the anti-HIV CAR T cell trafficking into tissues with viral replication in parallel by microscopy we developed an RNAscope-based RNA fluorescent in situ hybridization assay. To detect CAR mRNA, we designed probes against the single chain variable fragment and the 3' untranslated region. SHIV1157ipd3N4 probes were directed against gag, pol, tat env and nef.

Results: We validated the probes in vitro in primary macaque cells and in situ in different tissues of SHIV1157ipd3N4-infected pigtail macaques infused with HIVCAR T cells and in xenografts of anti-CD19 CAR T cell-infused mice.

Conclusions: The development of this RNAscope-based tool set allows us to study the interaction of anti-HIV CAR T cells and the HIV/SIV/SHIV reservoir and thus, to improve CAR T cell design for future interventions. The rational design of our detection system can be easily adopted to other gene therapeutic approaches in HIV and cancer.

PP 7.5

Clonotypic differences in TCR reactivity to HIV-1 Gag TL9 in the context of HLA-B*42 and HLA-B*81

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Background: HLA-B*81 is associated with control of HIV-1 subtype C infection, whereas the highly similar HLA allele B*42 is not. We observed that some B*42 and B*81 patients elicit CTL that recognize Gag TL9 presented on both B*42 and B*81; and that this phenotype

correlates with lower plasma viremia in B*42 individuals. Since peptide-HLA recognition is mediated by the TCR, we studied TL9 specific TCR repertoires in mono- and dual-HLA reactive CTL populations.

Methods: Single TL9-tetramer+ CTL were isolated by FACS from 6 patients (3 B*81, 3 B*42) displaying dual-reactive phenotypes. TCR beta genes were amplified by RT-PCR and sequenced. Paired TCR alpha genes were amplified for selected dominant clones. Full-length alpha/beta genes were synthesized and functionally assessed by transfection of Jurkat T-cells with CD8-alpha and an NFAT-driven luciferase reporter. TCR+ Jurkat 'effector' cells were co-cultured with B*81 or B*42 'target' cells pulsed with either TL9 or alanine variants or infected with HIV-1. TCR signaling was quantified by luminescence.

Results: 798 TCR beta sequences were collected from 6 individuals (avg. 67 per CTL population). In B*81 patients, dominant TCR beta genes were restricted to TRBV12-3 for mono- (B*81 TL9) and dual-reactive (B*81/B*42 TL9) cells. In B*42 patients, beta genes were variable for the mono-reactive (B*42 TL9) repertoire, while dual-reactive cells were restricted to TRBV12-3 and contained several public clonotypes. Paired TCR alpha/beta genes were reconstructed for selected clones representing each phenotype and peptide/HLA reactivity was confirmed using a reporter T-cell assay with peptide-pulsed or virus-infected targets. Signaling by mono-B*42 reactive TCR was more dependent on TL9 positions 2 (proline) and 3 (glutamic acid), compared to mono-B*81 and dual-B*42/B*81 reactive TCR clones, indicating that they are more sensitive to viral escape variants at these positions.

Conclusions: Analysis of the sequence and function of HIV-1 Gag TL9-specific TCR from B*42 and B*81 individuals revealed unique features of mono- and dual-HLA reactive phenotypes that may contribute to differential clinical outcome. Results highlight the potential impact of TCR repertoire on HIV-1 infection, and suggest that TCR clonotype should be considered during the development of novel vaccines and therapeutic strategies to prevent or cure HIV.

PP 7.6

Mobilizing NK cells for an HIV Cure: NK cells can target and kill latently HIV-1-infected primary T cells following proviral reactivation

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Background: Antiretroviral therapy does not cure HIV, due to the persistence of latent reservoirs, and latency-reversing agents (LRAs) as monotherapy do not reduce latent reservoirs due to an insufficient cellular immune response. Our approach to HIV eradication combines adoptive transfer of cytotoxic NK cells combined with antibody-dependent cell-mediated cytotoxicity (ADCC). Since NK cells innately recognize ligand density changes on infected target cells rather than any prior antigen sensitization, they are ideally suited to target cells infected with CTL-escape variants. Furthermore, downregulation of MHC-I by HIV Nef expression during HIV reactivation primes target cells for recognition by NK cells.

Methods: Cytotoxic NK cells were purified and expanded (eNK cells) from a cohort of well-suppressed HIV$^+$ participants on ART using artificial antigen presenting cell (aAPC) technology. Using models of acute and latent infection, we evaluated eNK cell-mediated killing of autologous HIV-1 infected primary T cells by flow cytometry and time-lapse microscopy. We then screened 24 α-HIV Env broadly neutralizing antibodies (bNAb) for their ability to mediate ADCC. Killing of memory T cells (Tm) from HIV$^+$ participants was detected by loss of inducible cell-associated HIV mRNA and proviral DNA.

Results: Culture of NK cells from HIV+/– participants with aAPCS led to expansion of (CD56^{bright}CD16$^+$) eNK cells that express multiple NK cell-activating receptors. In our acute and latent models with autologous primary T cells, eNK cells specifically killed HIV-infected cells while sparing the uninfected cells. eNK cells also maintained their ability to utilize anti-Env bNAbs to mediate ADCC against different HIV isolates, and bNAbs targeting the CD4 binding site were most effective. Using our HIV latency model in Th17 cells, we showed that IL-15 in combination with vorinostat optimally reactivates HIV–1 and induces Env expression. With this LRA treatment, we have been able to show that eNK cells from HIV$^+$ participants efficiently and progressively kill autologous HIV$^+$ Tm cells.

Conclusions: In conclusion, eNK cells show promise as effectors for clearing HIV reservoirs. Adoptive transfer of eNK cells into LRA-treated patients may represent an important new approach to virus eradication that can be rapidly translated into clinical use.

PP 7.7

SMAC mimetics reverse HIV latency by selective activation of the non-canonical NF-κB pathway

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Background: To date, success has been limited in finding a latency-reversing agent (LRA) that reactivates a large portion of the quiescent provirus without significant off-target effects. SMAC mimetics (SMACm) were recently identified as novel LRA (Pache, *et al*, 2015) and we have expanded upon the foundational work to identify a SMACm, AZD5582, with single agent LRA activity in resting CD4 T cells. Here we confirm the mechanism-of-action of SMACm to activate the non-canonical NF-κB (ncNF-κB) pathway and detail the transcriptional changes induced by SMACm.

Methods: Activation of latent HIV-1 was first tested with a Jurkat luciferase reporter model and verified by measuring cell associated gag RNA using RT-qPCR in *ex vivo* treated total and resting CD4 T cells from stably treated patients. Cell line and primary cell extracts were immunoblotted to test ncNF-κB activation. SMACm treated CD4 T cells were further analyzed by RNA-seq.

Results: We first verified that SMACm activate the ncNF-κB pathway as exemplified by depletion of cIAP1/2 and cleavage of p100 to p52. SMACm treatment of CD4 T cells led to rapid degradation of cIAP1/2 within 30 minutes of administration followed by a slower conversion of inactive p100 into active p52. Even a short pulse of AZD5582, similar to the expected *in vivo* exposure, lead to potent and durable ncNF-κB pathway activation lasting at least 48 hours. Comparison of gene expression changes between AZD5582 and PKCa-treated CD4 T cells confirm the slow but durable transcriptional activation by SMACm. Furthermore, AZD5582 led to a change in nearly 10-fold fewer genes than PKCa, with the only significant overlap with PKCa occurring in the NF-κB pathway signature.

Conclusions: In this study, we have confirmed that HIV latency reversal induced by SMACm is associated with ncNF-κB pathway activation. The activation kinetics of the ncNF-κB pathway are considerably slower than pathways triggered by other LRA but signaling is highly durable with pathway activation lasting several days. Moreover, RNA-seq analysis showed SMACm regulate a smaller number of genes as compared to PKCa. Together, these data support the continued evaluation of SMACm as an LRA in additional *in vitro* and *in vivo* model systems.

PP 7.8

Effect of tyrosine kinase inhibitors on the cytotoxic activity against HIV-1 infection

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Background: Both natural (NKs) and adaptive immune responses (CTLs) contribute to control HIV-1 infection but they are overcome by viral escape mechanisms. These cytotoxic cells cannot destroy the latently infected cells unless they get visible through activation. Several attempts to 'kick and kill' the reservoir have been implemented but, besides the reservoir reactivation, is necessary to induce the cytotoxic potential of the immune cells. Tyrosine kinase inhibitors (TKIs) such as imatinib and dasatinib used for treating chronic myeloid leukemia (CML), may interfere with HIV-1 infection. Besides, they are known to increase the number of NK (CD3-CD56+), NK-LGL (CD56+CD57+), and T-LGLs (CD3+CD57+) cells in CML patients and to decrease the number of Tregs. In this work, we analyzed the ability of TKIs to activate the cytotoxic cells and destroy HIV-infected cells.

Methods: CD4+ T cells isolated from PBMCs of healthy donors (n=6) and activated with PHA/IL-2 for 3 days were co-cultivated with their own cytotoxic (NK, CD8+) cells, previously treated with imatinib 10 uM or dasatinib 37.5 nM, and then infected with NL4-3_Renilla clone. The same procedure was performed with PBMCs from patients with CML treated with dasatinib (n=6) or imatinib (n=6), except for the treatment in vitro with TKIs.

Results: 1) Number of CD56+CD16+ cells was increased in PBMCs treated in vivo and in vitro with dasatinib and imatinib. 2)Basal synthesis of IFNg was increased 2.5- and 2.0-fold, respectively, in PBMCs of CML patients treated with dasatinib or imatinib, and 5.1- and 2.0-fold, respectively, in response to CEF peptides. 3) CML patients treated with dasatinib showed 2.2- and 2.1-fold increase of CD3+CD8+low and CD3+CD8+CD69/25+ cells, respectively, regarding healthy controls. Treatment with dasatinib also increased the percentage of TCRgamma/delta+ lymphocytes. 4) Cytotoxic activity against HIV-1 infection ex vivo was increased in PBMCs from CML patients on chronic treatment with dasatinib. These results were reproduced when PBMCs were also treated with dasatinib in vitro.

Conclusions: TKIs such as imatinib and dasatinib may increase the cytotoxic activity of NK and CD8+ T cells in vivo and in vitro. Therefore, treatment with TKIs could destroy latently infected CD4+ T cells, once the provirus is activated.

PP 7.9

SMAC mimetics are potent latency reversal agents with single agent and combination activity *ex vivo*

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Background: HIV latency reversal has thus far required potent stimulatory molecules or epigenetic modifiers to achieve meaningful responses *in vitro* or *in vivo*. However, these mechanisms have potentially broad off-target effects that limit their clinical applicability. We sought to identify a mechanism to reverse HIV latency that had minimal collateral impact and to this end focused on selective activation of the non-canonical NFkB pathway using SMAC mimetics, a class of investigational oncology drugs previously shown to reactivate HIV latency in model systems by Pache, et al., in 2014.

Methods: We studied a range of SMACm for latency reversal activity *in vitro* using latent HIV-infected Jurkat cells with a luciferase reporter, CD4+ T cells infected *ex vivo* with an HIV reporter virus, and HIV gag RNA induction in patient-derived CD4+ T cells.

Results: A diverse set of SMACm activated our latently HIV-infected Jurkat cell model with a range of potency and maximum responses. SMACm-induced Jurkat signal increased over 48 hours of stimulation, in agreement with slower kinetics of ncNFkB as compared to more rapid canonical NFkB activation. HIV latency disruption was also observed in reporter-based primary cell models and in patient-derived resting CD4+ T cells in response to the most potent molecule tested here, AZD5582, but not less potent molecules such as birinapant. These latency reversal activities are observed in the absence of induction of T cell activation markers, indicating that the activity of SMACm does not lead to full cellular activation. Importantly, short exposures to pharmacologically achievable concentrations of AZD5582 led to HIV gag RNA induction in *ex vivo* patient-derived CD4+ T cells. In addition, SMACm synergized with BETi and HDACi in the Jurkat reporter cells and patient-derived cells.

Conclusions: We confirm that SMACm potently induce HIV RNA from model systems and show for the first time that SMACm have single agent latency reversal activity in *ex vivo* patient-derived CD4+ cells. Importantly, this activity can be engaged with concentrations and durations of exposure that are achievable in preclinical models. These findings support progression of SMACm to testing in animal models as clinically relevant and selective latency reversing agents.

PP 7.10

Novel use of alprazolam as a potential HIV-1 latency reversing agent

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Background: The mechanism that controls HIV-1 latency is not fully understood. Our lab has shown previously that RUNX1, a host transcription factor; may play a role in establishment and maintenance of HIV-1 latency. We also previously showed that the benzodiazepine (BZD) RUNX1 inhibitor, RO5-3335 synergizes with SAHA in reversing HIV-1 latency. In order to find putative RUNX1 inhibitors, we screened clinically prescribed BZDs for their ability to reverse HIV-1 latency. We hypothesize that some FDA approved BZDs will synergize with SAHA to activate HIV-1 latency.

Methods: J-Lat 10.6 cells were treated with different doses of BZDs and the percentage of GFP positive/ live cells was measured by flow cytometry. Primary PBMC from healthy donors were used to investigate whether alprazolam (Xanax) is specifically activating HIV-1 or having a general effect on T cell activation (CD4 and CD8). T-cell activation was calculated as the percentage of CD3+/CD4+ or CD3+/CD8+ cells that were CD69+. To investigate whether alprazolam has an inhibitory effect on RUNX1, expression of RUNX1 responsive genes was determined by qPCR.ChIP on TZM-bl cells was used to look at the mechanism of alprazolam-based reactivation.

Results: Alprazolam showed a strong HIV-1 reactivation at 10 µM (20% higher than the DMSO control) with high bioavailability in the presence or absence of SAHA when tested on Jlat 10.6. Interestingly, alprazolam in combination with SAHA did not induce any significant T cell activation. We also found that alprazolam significantly affects some RUNX1 responsive genes in a trend similar to RUNX1 inhibitor, Ro5-3335. Moreover, alprazolam increases the CBP/P300 level and it synergizes with SAHA to increase the level of H3k9ac at HIV-1 promoter.

Conclusions: Overall, our findings identified alprazolam as a potent latency-reversing agent.

PP 7.11

HIV-1 Nef dimeriation and AP-2 recruitment contribute to viral replication and T-cell loss in humanized mice

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Background: The HIV-1 Nef accessory factor enhances viral infectivity, persistence, and AIDS progression. Nef hijacks host cell trafficking pathways to downregulate membrane-bound receptors, including CD4 and MHC-I, to promote immune escape. We have shown that small molecule inhibitors targeting the Nef dimerization interface promote CD8 T cell-mediated elimination of latently HIV-infected CD4 T cells in vitro, partly due to reversal of MHC-1 downregulation by Nef. We have also shown that Nef dimerization is important for AP-2 adaptor recruitment and CD4 downregulation, which enhances viral infectivity and immune escape from ADCC. These findings point to Nef as a rational target for antiretroviral drug discovery.

Methods: To test the role of Nef in HIV-1 replication and CD4 T cell depletion in vivo, we used the BLT (bone marrow-liver-thymus)

and hu-PBMC-NSG humanized mouse models. Mice were infected with HIV-1 NL4-3 harboring wild-type Nef, dimerization- and AP-2-binding-deficient Nef mutants, as well as a Nef-deleted virus. We measured viral loads in mouse plasma by real-time quantitative RT-PCR (qPCR) and human T cell counts by flow cytometry. We also quantitated viral tissue infiltration using qPCR targeting HIV Gag or by p24 alpha assay.

Results: Humanized PBMC-NSG mice infected with Nef-deleted HIV-1 or the Nef dimerization-deficient mutant developed less severe viremia and decreased viral loads in spleen and liver compared to controls infected with wild-type virus. Mice infected with these Nef mutants also displayed CD4 T cell counts comparable to uninfected mice. Two-thirds of mice infected with HIV-1 carrying a Nef mutant (Asp 174/175 to Ala) deficient for AP-2 binding also displayed normal CD4 T cell counts. Similarly, BLT mice infected with macrophage-tropic HIV-1 p81a with wild type Nef had lower peripheral T cell counts compared to mice infected with the Nef-deleted virus.

Conclusions: Data from HIV-infected humanized mice support the importance of Nef expression, dimerization, and interaction with AP-2 for efficient HIV-1 replication and CD4 T cell loss in vivo. Ongoing studies will address the role of Nef dimerization and AP-2 recruitment in latent viral infection and the possibility of pharmacological suppression of these Nef functions using humanized mouse models.

PP 7.12

Adoptive T cell as a strategy for targeted delivery of immune checkpoint therapy

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Background: The HIV specific CD8-T cell response to HIV is impaired by immune checkpoint expression, such as PD-1.Although systemic use of PD-1 blockade therapy has been revolutionary in the oncology field and preclinical studies show promise of PD-1 blockade in the HIV setting, the high rates of induction of immune related adverse events (irAEs) renders systemic administration of immune checkpoint inhibitors to ART suppressed, HIV-infected individuals problematic. A more targeted approach to harness the benefits of immune checkpoint inhibitor therapy for HIV eradication studies without the risk for irAEs that comes with systemic, non-specific, administration is urgently needed. Immune checkpoint blockade during ex-vivo expansion of HIV-specific T cells may enable expansion of a population of previously expansion-resistant, highly avid PD-1 expressing HIV-specific CD8-T cells with enhanced antiviral capacity against latent HIV infection, allowing us to harness the power of immune checkpoint blockade in a more targeted and safe manner.

Methods: PBMCs from ART-suppressed HIV-infected individuals are isolated from leukapheresis products and HIV-specific T cells are expanded in the presence of gag peptides, IL-7, IL-12, IL-15, and IL-2 as well as in the presence or absence of 10 μg/ml anti-PD-L1 antibody or isotype antibody. Antiviral frequency and magnitude is assessed by IFNγ ELIspot assay. Antiviral capacity against both JR-CSF and autologous reservoir virus is assessed by a viral inhibition assay.

Results: CD8-T cells from ART suppressed HIV infected individuals show improved antiviral capacity with PD-1 blockade ($n=7$, $p<0.01$ by Wilcoxon signed rank test). This is the first use of PD-1 blockade in a viral inhibition assay to assess impact on antiviral capacity. Preliminary data shows PD-1 blockade during ex-vivo expansion yielded 2 times greater fold expansion of HIV-specific T cells compared to isotype antibody, and enhanced antiviral capacity ($p<0.05$ by One way ANOVA with Dunnett's post-test correction for multiple comparisons).

Conclusions: Preliminary data suggests PD-1 blockade during ex-vivo expansion of HIV-specific T cells may yield T cells with greater antiviral capacity in-vitro. Ultimately, adoptive T cell therapy could provide a vehicle for targeted delivery of immune checkpoint therapy. Further studies will be necessary to validate this approach.

PP 7.13

HIV-specific T cells expressing an X5-GPI artificial receptor can suppress hiv replication *in vitro*: implications for a cure strategy for HIV-positive individuals with hematologic malignancies

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Background: Multi-antigen HIV-specific T-cells (HXTC) derived from both HIV+ and HIV negative individuals, suppress active HIV replication in vitro – providing a promising platform for mediating a functional HIV cure in the context of allogeneic stem cell transplant. While the inclusion of CD4+ T-cells in T-cell products has been shown to enhance in vivo persistence, in the HIV setting, the risks for infection is a limitation. We hypothesized that HXTCs can be rendered resistant to HIV infection by genetically modifying the HXTC to express to a broadly neutralizing HIV antibody directed against the X5 portion of HIV envelope via a GPI (glycosyl-phosphatidylinositol)-AR (artificial receptor). The GPI signal targets the X5-scFv for expression on lipid rafts, placing it in close proximity to CD4. Hence, we posited that during HIV infection of a CD4 T-cell, the X5 GPI-AR would recognize the transiently exposed X5 epitope on gp120, blocking the fusion step.

Methods: We transduced HXTC derived from HIV negative (HIVneg, n=5) and HIV positive (HIV+, n=9) donors with a retrovirus encoding X5-GPI AR.

Results: Expression of X5 was measured using a poly-his tag on the GPI-AR with mean expression of 60.45% (HIV+, n=8) and 58.33% (HIV negative, n=4). No difference in HXTC expansion was observed (n=8). Further, we found no change in HIV specificity, as measured by IFNγ-ELISPOT in non-transduced HIV+ or HIVneg HXTC (576 and 376 SFC/105 cells respectively) versus transduced HXTC (601 and 382 SFC/105 cells respectively). No difference in the expression of exhaustion markers between non-transduced and X5-transduced HXTC was observed. Additionally, preliminary data suggests that HXTC expressing X5 GPI-AR may suppress HIV replication in autologous HIV-infected CD8neg T-cells more than unmodified HXTC or nonspecific-T-cells expressing the X5 GPI-AR, as measured in viral inhibition assays (n=4).

Conclusions: We conclude that X5 GPI-AR expressing HXTC have superior potency in vitro than unmodified HXTC. We plan to integrate the X5-scFv into a CAR to genetically modify HXTCs and propose that X5-CAR expressing HXTCs could be a potent therapeutic for the treatment of HIV+ individuals post-SCT, targeting multiple HIV antigens while preventing infection of infused CD4+ T-cells.

PP 7.14

HIV protease cleavage sites vaccine augments quality of T cell responses during ART

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Background: An effective therapeutic HIV vaccine for use in combination with combination anti-retroviral therapy, could limit latent reservoir establishment, promote viral suppression and improve immune

responses in HIV infected individuals. We conducted an evaluation of the efficacy of a novel therapeutic HIV vaccine targeting the 12 protease cleavage sites (PCS) administered in combination with ART.

Methods: SIVmac251 infected Rhesus monkeys were treated with a combination of FTC, PMPA and raltegravir for 49 days. Seven days after ART initiation, one group of monkeys received recombinant VSV-PCS intramuscularly, followed by 3 therapeutic vaccinations with rVSV-PCS and NANOpcs (i.m) at 2-week intervals. PBMCs purified collected at each immunization time point, was separately stimulated with pooled peptides for PCS, Gag or Env overnight. Multicolour Flow cytometry analysis was used to evaluate T cell responses in antigen stimulated and unstimulated PBMCs from controls (ART only) and vaccinated macaques, while cell-culture supernatants was assessed using multiplex assays for soluble cytokines and chemokines.

Results: Ex vivo evaluation of immune activation and cytokine production by T cells in ART treated and vaccinated macaques, revealed a lower expression of chronic activation marker CD38+ but higher frequencies ofIFNγ, IL-2, MIP1β and TNFα producing cells compared to control animals. The higher cytokine reponses by CD4+ T cells in vaccine treated macaques was neither accompanied by alterations in cellular activation nor T cell exhaustion. The capacity of CD4+ T cells to degranulate based on CD107a expression correlated with frequencies of CD4+ T cells in vaccinated but not in ART only treated macaques. Antigen stimulation of PBMCs of ART and rVSVpcs/NANOpcs vaccine treated macaques did not significantly increase CD4+ T cell activation based on CD69 expression, but resulted in higher frequencies of CD8+IFNγ+ and CD8+CD69 T cells. Finally, multiplex quantification of cytokine and chemokine responses in PCS peptide primed PBMCs from vaccinated animals, revealed a higher TNFα and GM-CSF responses.

Conclusions: These results suggest that rVSVpcs/NANOpcs vaccination of SIV infected macaques during ART, improved the quality of CD4+ and CD8+ T cell responses, than ART only, and offers hope for the development of therapeutic HIV vaccines targeting to improve the quality of immune responses.

Session 8: New therapeutic approaches II (oral presentations only)

OP 8.1

HIV-specific T cells generated from HIV-naive adult and cord blood donors target a range of novel viral epitopes: implications for a cure strategy after allogeneic HSCT and CBT

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Background: Adoptive T cell therapy has been successful in boosting viral-specific immunity post-HSCT, preventing viral rebound of CMV and EBV. However, the therapeutic use of T-cells to boost HIV-specific T-cell immunity in HIV+ patients has been met with limited success. Despite multiple attempts to eradicate HIV with allogeneic HSCT, there is only one case of functional HIV cure. Hence, we hypothesized that broadly HIV-specific CD8 and CD4 T-cells (HXTCs) could be expanded from patients on ARVs, as well as HIV negative adult and cord blood donors (dHXTC), employing a non–HLA restricted approach for the treatment of HIV+ individuals after auto or allo-HSCT.

Methods: We have expanded autologous HXTCs from HIV+ subjects under NCT02208167. To extend this approach to the allo-HSCT setting, we generated dHXTCs from HIV naive adults (n=8) and cord blood (CB) donors (n=8).

Results: IFNg-ELISPOT showed dHXTCs from adult donors were specific against Gag (mean=331.25 SFC/105cells) and Nef (mean=242.63) versus actin (mean=13 SFC)(n=8). Similarly, we are able to produce cord dHXTCs (n=8) that showed specificity to Gag (mean=103.252 SFC/105cells), Nef (mean=109.43), or Pol (mean=130.56), ($p<0.0001$ compared to controls) in IFNg-ELISPOT. dHXTCs were polyfunctional, producing pro-inflammatory TNFα, IL2, IL6, IL8, and perforin responses ($p<0.05$) to HIV stimulation. Importantly, dHXTCs derived from both adult ($p=0.0004$) and CB ($p=0.0003$) were able to suppress HIV replication compared with nonspecific CD8+ T-cells when co-cultured with autologous CD4 T-cells infected with HIV SF162 at an E:T ratio of 20:1. Exhaustion marker analysis of CB dHXTC lines revealed minimal expression of PD1, TIM3, LAG3, and KLRG1 (n=3). Epitope mapping revealed that dHXTC products contained T-cells recognizing unique epitopes not typically identified in HIV+ individuals, which may be critical in overcoming viral immune escape post-HSCT.

Conclusions: In summary, HIV-specific T-cells can be expanded from HIV+ and HIVneg donors for clinical use. Focusing on donors with HLA types that are associated with well characterized HIV responses (HLA A02) may allow us to identify HLA restricted epitopes critical for the successful development of a potent HIV-specific T-cell therapeutic. Hence, the administration of dHXTCs derived from naive donors could offer a unique curative strategy post-allogeneic HSCT and CB transplant.

OP 8.2

Oral ABX464 reduces the HIV DNA reservoir IN CD4+ peripheral blood T cells

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Background: ABX464, first-in-class orally available small molecule that blocks HIV replication by inhibition of rev activity. Preclinical data in HIV-1 infected humanized mice showed that ABX464 monotherapy had an antiviral effect that was sustained following treatment interruption. In clinical studies, ABX464 has shown dose-dependent reduction in HIV-1 RNA in treatment-naïve study participants, and decrease in total HIV-1 DNA in peripheral blood mononuclear cells (PBMC) following 28 days of daily oral dosing in virally suppressed individuals. The current study, ABX464-005, is an ongoing Phase 2a clinical trial investigating the safety, pharmacokinetic (PK), and pharmacodynamic (PD) profile of ABX464.

Methods: Healthy HIV-1 infected male participants with CD4 nadir ≥250 cells/mm3 and plasma HIV-1 RNA ≤100,000 copies per ml were eligible to enrol. Participants took 150 mg oral ABX464 for 28 days with blood, and rectal biopsy samples taken by flexible sigmoidoscopy, at Baseline and Days 28 and 56 to quantify PK and PD (HIV-1 DNA in CD4+ T cells and in rectal biopsy CD45+ mucosal mononuclear cells (MMC)). HIV-1 DNA in was quantified in CD4+ T cells and CD45+ MMC lysates using digital droplet PCR as previously described (PLoS ONE 12(4):e0175899).

Results: Eleven participants were recruited and 2 withdrew due to adverse events (1 with abdominal pain (grade (G)1), headache (G1), hyperamylasemia (G2), and hyperlipasemia (G2), 1 with headache, myalgia, asthenia, insomnia and backache (all G1). There were no serious adverse events. One participant did not have rectal biopsies at Day 28 and 56 due to flare of pre-existing haemorrhoids. CD4+ T cell HIV-1 DNA fell from median of 191.1 (interquartile range (IQR) 80.1-368.1) to 116.0 (97.4-1185.0) copies/106 between Baseline and Day 28, respectively ($p=<0.01$) (Figure 1). There was no significant difference in CD4+ T cell HIV-1 DNA between Days 28 and 56. The variability in yield of MMC obtained from the rectal biopsies, precluded analysis of MMC HIV-1 DNA.

Conclusions: Exposure to ABX464 was associated with significant decrease in HIV-1 DNA in CD4+ T cells. Overall, these data confirm the decrease in PBMC HIV-1 DNA seen in the ABIVAX-004 study and support the continued development of ABX464 as a component of cure eradication strategies.

OP 8.3

Interim safety analysis of cancer immunotherapy trials Network – 12 (CITN-12): a Phase 1 study of pembrolizumab in patients with HIV and cancer

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Background: Anti-PD-1 and anti-PD-L1 antibodies are becoming mainstays of cancer therapy. The safety of pembrolizumab, an anti-PD-1 humanized monoclonal antibody, is being evaluated in patients with HIV and cancer. The effect of anti-PD-1 therapy on HIV reservoirs is unknown.

Methods: CITN-12 is a multicenter study of pembrolizumab in patients with HIV and advanced cancers. Three CD4 defined cohorts (C) are accruing; C1: 100-199, C2: 200-350, and C3: >350 cells/uL. Eligibility: >4 weeks antiretroviral therapy (ART), HIV viral load <200 copies/mL. Treatment: pembrolizumab 200 mg intravenously every 3 weeks for up to 2 years. Primary objective: assess safety and tolerability by summarizing CTCAEv4 graded adverse events (AEs) and evaluating HIV viral load (VL) and CD4 counts. Immune mediated AEs

are managed using standard guidelines. We performed an interim analysis of treatment emergent adverse events at least possibly related to pembrolizumab (rTEAEs), serious AEs, and CD4 counts on therapy. Plasma HIV VL was measured by an HIV gag single copy assay (SCA).

Results: 17 patients were accrued starting April 2016 and followed through May 2017. Characteristics: 1 woman, 16 men; median age 56 years (range 43-77); Cancers: lymphoma (3), Kaposi sarcoma (1), anal (5), tonsil (1), lung (2), bladder (1), hepatocellular (1), pancreatic (1), cholangiocarcinoma (1). Safety was observed over 100 total cycles, median 4 (range 1-20). 82 rTEAEs were observed and comparable between cohorts. 93% were grade 1-2. Ten primary serious AEs were observed, 2 possibly attributable to pembrolizumab, both in the setting of progressive malignancy. Immune mediated AEs: subclinical hypothyroidism 6 (35%), pneumonitis (2) and liver test elevations (2). Median CD4 increased over time, changes did not reach statistical significance. HIV remained suppressed on ART in all patients. In a subset of 14 patients, baseline median HIV VL by SCA was 0.8 copies/mL (range: <0.3-9.9); no significant increases between noted.

Conclusions: Pembrolizumab has an acceptable safety profile to date in patients with cancer and suppressed HIV on CITN-12, with no evidence of increased HIV VL over 6 weeks of therapy. Anti-PD1 therapy is appropriate for FDA approved indications in HIV-infected patients. Studies evaluating HIV latency reversal, HIV-specific immunity, and HIV reservoirs are underway.

OP 8.4

Direct and indirect effects of synthetic dual TLR-2 and TLR-7 agonists (Dual TLR-2/7) on latent HIV

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Background: Toll-like receptor (TLR) agonists induce antiviral immune responses and have the ability to reactivate latent HIV. Our group has shown that TLR-2 agonists can directly reactivate latent HIV in resting CD4 T cells. On the other hand, TLR-7 agonists reactivate latent HIV in an indirect manner through the production of stimulatory cytokines by plasmacytoid dendritic cells. TLR-7 agonists also promote antiviral immune responses. In this study, we have characterized the ability of three novel synthetic TLR ligands comprised of a TLR-2 and a TLR-7 ligand (Dual TLR-2/7) to trigger antiviral immune responses and viral reactivation.

Methods: We first analyzed the ability of four dual TLR-2/7 agonists to reactivate latent HIV in a modified J-Lat clone that expresses TLR-2 in the surface. Three out of the four retain their ability to reactivate latent HIV and were analyzed for their ability to produce anti-viral and pro-inflammatory cytokines, immune activation, and latency reversal in a primary cell model of HIV-1 latency and PBMCs from aviremic HIV participants.

Results: We have found that these novel synthetic dual TLR-2/7 agonists retain the ability to directly reactivate latent HIV in CD4 T cells through TLR-2 by inducing NF-kB activation. Moreover, they also induce HIV reactivation in PBMCs isolated from aviremic participants. Furthermore, these agonists induce production of pro-inflammatory and anti-viral cytokines, as well as T cell and NK cell activation in a similar fashion as TLR-7 agonists.

Conclusions: Our data indicate that synthetic dual TLR-2/7 agonists maintain both the ability to directly reactivate latent HIV in CD4 T cells and to promote antiviral immune responses. These combined effects may favor the clearance of the latent reservoirs and suggest further exploration of these agonists for HIV eradication studies.

OP 8.5

Partial control of viral rebound with a Rev-dependent lentiviral vector carrying HSV-tk gene in SIV-infected rhesus macaques

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Background: Persistence of HIV in anatomic sanctuary sites such as the brain prevents viral eradication. Although combination antiretroviral therapy (cART) inhibits viral replication to undetectable level, it does not selectively eliminate virus reservoirs. To target HIV reservoirs, we have developed an HIV Rev-dependent lentiviral vector carrying a series of therapeutic genes, such as diphtheria toxin, anthrolysin O from Bacillus anthracis, human TRAF6, or the herpes simplex 1 virus thymidine kinase gene (HSV-tk). We have tested the Rev-dependent vectors for Rev-dependent selective expression and killing of HIV-infected cells in vitro.

Methods: Recently, we further tested the feasibility of using the Rev-dependent vector to target viral reservoirs in a SIV/rhesus macaque model. SIV-infected rhesus macaques were first treated with cART for over 6 months starting 12 weeks post infection, followed by injections with viral particles assembled from a SIV Rev-dependent vector carrying HSV-tk. Following particle injection, animals were treated briefly (two weeks) with ganciclovir (GCV), which induces the killing of SIV+, HSV-tk expressing cells. cART was terminated following the GCV treatment.

Results: We observed a partial control of viral rebound with this single vector treatment scheme over a period of 4 months after cART cessation.

Conclusions: Our results suggest that the Rev-dependent vector has the potential to diminish viral reservoirs in vivo.

OP 8.6

Chronically treated HIV-positive subjects can naturally harbor extremely low viral reservoir

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Background: Small size viral reservoirs are predominantly found in HIV-1 controllers and subjects who received cART during primary HIV-1 infection. However, we have previously reported that about 9% of individuals treated during the chronic phase of infection are 'Low Viral Reservoir Treated' (LoViReT). Herein, we have characterized the reservoirs dynamics in those LoViReT individuals aiming to decipher natural factors to achieve HIV eradication.

Methods: LoViReT were defined as chronically treated individuals with <50 HIV-DNA copies/10^6 PBMCs. Longitudinal CD4+ T cells were obtained from 17 LoViReT individuals and compared to 17 controls (>50 HIV DNA copies/106 PBMCs under cART) chosen by using a random forest based matching method and considering relevant clinical factors. Total HIV-1 DNA was analyzed by ddPCR using two different primer sets, GAG and/or LTR.

Results: Retrospective longitudinal reservoir analysis in CD4+ T cells was performed in 34 subjects using a median of 4 samples per individual, including a pre-cART sample (Figure). We observed that LoViReT individuals initially harbored lower levels of HIV-DNA before treatment initiation (LoViReT: 812 [485–1392] vs. controls: 5994

[2653–8811] HIV-DNA copies/106 CD4+ T cells, p<0.001) despite similar viral loads between both groups. After treatment introduction, most of the individuals showed a pronounced proviral reservoir reduction during the first 18 months, with a negative but softer decay afterwards. Overall, we observed that HIV reservoir decay was significantly greater in LoViReT subjects when compared with Controls (12 vs. 5.3 folds respectively, p<0.001).

After a period of 3 to 6 years of treatment initiation both groups showed the greatest differences within the study (LoViReT: 56 [37-89] vs. controls: 700 [548-1462] HIV-DNA copies/10^6 CD4+ T cells, p<0.001).

Conclusions: LoViReT individuals harbor an extremely low HIV-1 proviral load during suppressive chronic cART. A combination of natural reduced viral reservoir before cART with an enhanced latency decay after treatment initiation seems to be the cause of this exceptionally low reservoir. Further virological and immunological studies will decipher the mechanisms that contribute to the natural establishment of this otherwise more ART-sensitive viral reservoir.

Session 9: Pharmacology and drug discovery (poster presentations only)

PP 9.0

Role of mitochondrial antiviral signaling protein in reactivation of latent HIV-1 in CD4+ T cells

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Background: The mitochondrial antiviral signaling protein (MAVS) is involved in response to viral infection, and mediates signaling that culminates in type I interferon production and activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). HIV-1 has NF-κB binding sites in its long terminal repeat, allowing it to use NF-κB for viral transcription. We hypothesize that stimulation of MAVS in CD4+ T cells can reactivate latent HIV-1, and investigated the potential of targeting MAVS as a latency reversing strategy.

Methods: To assess MAVS stimulation as a potential strategy for reactivation, we have used a primary CD4+ T cell model which recapitulates latency in vitro, as well as resting CD4+ T cells from aviremic participants. We also employed a CRISPR/Cas9 strategy to study the role MAVS plays in viral reactivation using the latent HIV-1 model JLAT 10.6.

Results: We have shown that chemical activation of MAVS, triggered by reactive oxygen species, or endoplasmic reticulum (ER) stress is sufficient to reactivate latent HIV-1. Interestingly, we have found that several widely studied latency-reversing agents (LRAs) mediate their effect partly through the activation of MAVS.

Conclusions: Our results show that targeting MAVS in latently infected HIV-1 cells can lead to viral reactivation. Thus, MAVS is a potential therapeutic target for reversing HIV-1 latency.

PP 9.1

Synergistic HIV latency reversal from an *in vitro* screen of epigenetic and kinase inhibitors

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This work was done in collaboration with the laboratory of Dr. David Margolis at the University of North Carolina at Chapel Hill HIV Cure Center.

Background: HIV latency is maintained in part by repressive chromatin structure. Prior studies have demonstrated synergistic reactivation of HIV transcription using epigenetic inhibitors in combination with mitogens. However synergistic latency reversing agents (LRA) of kinase inhibitors and chromatin-targeted agents have not been explored systematically. In this study we investigated the combination of epigenetic and kinase inhibitors to identify novel targets for latency reversal.

Methods: We used a Jurkat HIV-luciferase reporter expression system to screen single agents and combinations from two targeted, drug-like, and well-annotated small molecule libraries: the EpiG diamond set and the GSK published kinase inhibitor set (PKIS).Data were analyzed using a Bliss independence model (ANOVA $p<0.05$, >2 SD from zero).Synergistic LRA pairs were confirmed with extended combination matrices in the Jurkat model and subsequently tested for induction of HIV gag RNA in total CD4+ T cells from HIV-infected patients fully suppressed on antiretroviral therapy.

Results: Most epigenetic inhibitors displayed activity as single agents in the Jurkat model, whereas only a few PKIS compounds were weakly active. A pilot study confirmed that the Jurkat model was able to detect previously reported synergistic interactions. From a combination screen of a subset of EpiG with PKIS compounds we identified eleven synergistic pairs, all of which exhibited low magnitudes of synergy ($\Delta fa\_xy<0.2$). We confirmed synergy between the bromodomain inhibitor I-BET151 and two 3-amino pyrazolopyridazine kinase inhibitors in dose-response combination matrices. These inhibitor combinations were further shown to induce HIV RNA in patient-derived CD4+ T cells with a high magnitude of synergy.

Conclusions: These results suggest that the pyrazolopyridazine kinase inhibitors identified and I-BET151 are synergistic when used in combination for HIV latency reversal. Further studies are needed to define the kinases involved, investigate the mechanisms of synergy, and determine the suitability of these combinations for clinical studies of latency reversal.

PP 9.2

Novel mechanisms of baricitinib to block reservoir seeding and HIV persistence

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Background: Baricitinib is a Jak 1/2 inhibitor (JAKI), EU-approved for rheumatoid arthritis, and has advantages to other Jak inibitors including lower overall dosing, longer plasma half-life, no renal clearance, and ability to cross the blood-brain barrier.We evaluated its ability to block events in myeloid and lymphoid cells that may drive malignancies in HIV-infected individuals including 1) viral replication, 2) HIV-induced activation, 3) reservoir expansion, 4) reservoir maintenance.

Methods: CD4+ T-cells, monocytes, or macrophages (MΦ) were cultured from healthy uninfected donors.T-cells were infected with HIV-1LAI for 6 days prior to quantification of extracellular virus (p24-ELISA).Non-dividing p24+/Bcl-2+ and PD-1+ cells were quantified (FACS).MΦ and monocytes were infected with HIV-1BaL for 6 days +/− baricitinib prior to quantification of HLADR/CD163 and CD14/CD16.

Results: Baricitinib demonstrated nanomolar inhibition of viral replication in CD4+ T-cells and macrophages(EC50/90 0.007/0.01 μM and 0.009/0.3 μM, respectively). Baricitinib significantly reduced ($p<0.01$): non-dividing CFSEhi / p24+/ Bcl-2+ and PD-1+ T-cells (EC50/900.01/0.3 μM); HIV-induced HLA-DR/CD163+ MΦ (EC50/900.02/0.4 μM; HIV-induced monocyte activation (CD14/CD16+; EC50/900.01/0.1 μM); and HIV reactivation in CD4+ T-cells and MΦ (EC50/900.007-0.1/0.09-0.3 μM).

Conclusions: Key events that modulate systemic immune activation that can contribute to secondary malignancies in HIV-infected individuals are blocked by baricitinib at physiologic concentrations.Baricitinib significantly reduces markers of viral persistence, reservoir size, maintenance, and expansion in CD4+ T-cells and monocyte/macrophages.These data suggest that baricitinib could reduce events that modulate immune dysregulation and therefore is a viable candidate for consideration as an add-on therapy to mitigate HIV-induced immune dysregulation.

PP 9.3

Molecular characterisation of the inhibitor didehydro-Cortistatin A with the HIV-1 Tat protein

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Background: The HIV-1 Tat protein binds the HIV mRNA trans-activation response (TAR) element, recruits cellular transcriptional cofactors, and exponentially amplifies viral mRNA expression. There are no clinically available anti-Tat drugs. We reported that didehydro-Cortistatin A (dCA) inhibits Tat and viral production from integrated proviruses at nanomolar concentrations. dCA promotes a state of sustained latency, from which transcriptional reactivation is drastically diminished. Here, we characterized the interaction of Tat with dCA.

Methods: Isothermal titration calorimetry (ITC) determined the affinity of dCA for Tat. Structure-activity relationship (SAR) studies of dCA and 11 analogs synthesized, combined with molecular modeling using AutoDock Vina of Tat NMR data, revealed interacting chemicals groups of dCA mainly with Tat's basic patch residues. dCA specificity for Tat's basic domain was evaluated by electrophoretic mobility shift assay and chromatin immunoprecipitation (ChIP). We excluded interaction of dCA with proteins with similar basic domains (Hexim-1 and HIV-1 Rev), using immunofluorescence, qPCR and ChIP. Tat's basic domain is also responsible for the stability of the protein, and dCA's stabilization of Tat was demonstrated by Trp fluorescence and enzymatic degradation assays.

Results: Nanomolar affinity of dCA for Tat was determined by ITC. SAR revealed the importance of the nitrogen atom (position and orientation) in the isoquinoline group and the cycloheptene ring of dCA, in the interaction with Tat's basic (K^{50}, K^{51}, R^{53} and R^{55}) and N-terminal (P^3 and E^2) regions. We demonstrated that dCA disrupts Tat-TAR interaction and stabilizes Tat structure, potentially useful in Tat crystallization efforts.

Conclusions: We characterized the molecular determinants for the activity of dCA on Tat, which is invaluable for the clinical development of the Cortistatin A pharmacophore, and for the development of Tat inhibitors.

PP 9.4

Validation of an unbiased screen method for the identification of secondary fungal metabolites reversing HIV-1 latency

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Background: Latency reversal agents (LRAs) are of high demand for HIV-1 cure strategies, as it is believed that reversing HIV-1 latency will lead to eradication of the virus from the body. Fungi are the source of a diverse array of secondary metabolites that are secreted into the fungal growth environment. Here we propose a sequential fractionation coupled to NMR/Mass Spec strategy for the screening and identification of potential LRAs from fungal growth supernatants.

Methods: We tested culture supernatants from over 100 fungal species known to produce a wide diversity of metabolites for LRA using the J-Lat model system of HIV latency. To identify active components of supernatants with potential LRA, positive fungal supernatants were fractionated by means of an orthogonal LC method and the latency reversal activity of the single fractions was assessed in the J-Lat models and followed by NMR. The composition of fractions reactivating HIV latency was determined by NMR. The candidate latency reversal compounds were verified in the cell-line and primary cell model systems of latency. Additionally, we tested the impact of our strongest hit on effector T cell functions. In order to delineate mechanism by which our strongest hit reversed latency we performed RNA sequencing on primary CD4+ T cells stimulated for 4 hours with FS-74-7BC.

Results: Identified small molecule FS-74-7BC showed potent LRA and negligible impact on J-Lat cells viability. Moreover, FS-74-7BC strongly synergized with the spectrum of LRAs. FS-74-7BC induced HIV-1 expression in ex-vivo infected primary CD4+ T cells in a concentration-dependent manner, reaching over 13-fold activation. Moreover, although slightly reducing the effector functions of CD4+ and CD8+ T cells, FS-74-7BC was not toxic to the primary cells nor did it induce T cell activation. Finally, RNA sequencing revealed that the most downregulated transcript was RN7SK.

Conclusions: Combination of mycology, state-of-art NMR spectroscopy and relevant bioassays proved to be powerful approach to identify potential novel LRAs. FS-74-7BC demonstrated potent HIV-1 latency reversal, which was synergistically enhanced by known LRAs. RNA sequencing data indicated that treatment with FS-74-7BC possibly results in dissociation of 7SK snRNP complex and subsequent release of pTEF-b. FS-74-7BC will be further investigated in CD4+ T cells isolated from aviremic HIV+ patients. Additionally, immunoprecipitation and glycerol gradient experiments on 7SK snRNP complex will be performed to confirm the mechanism of action.