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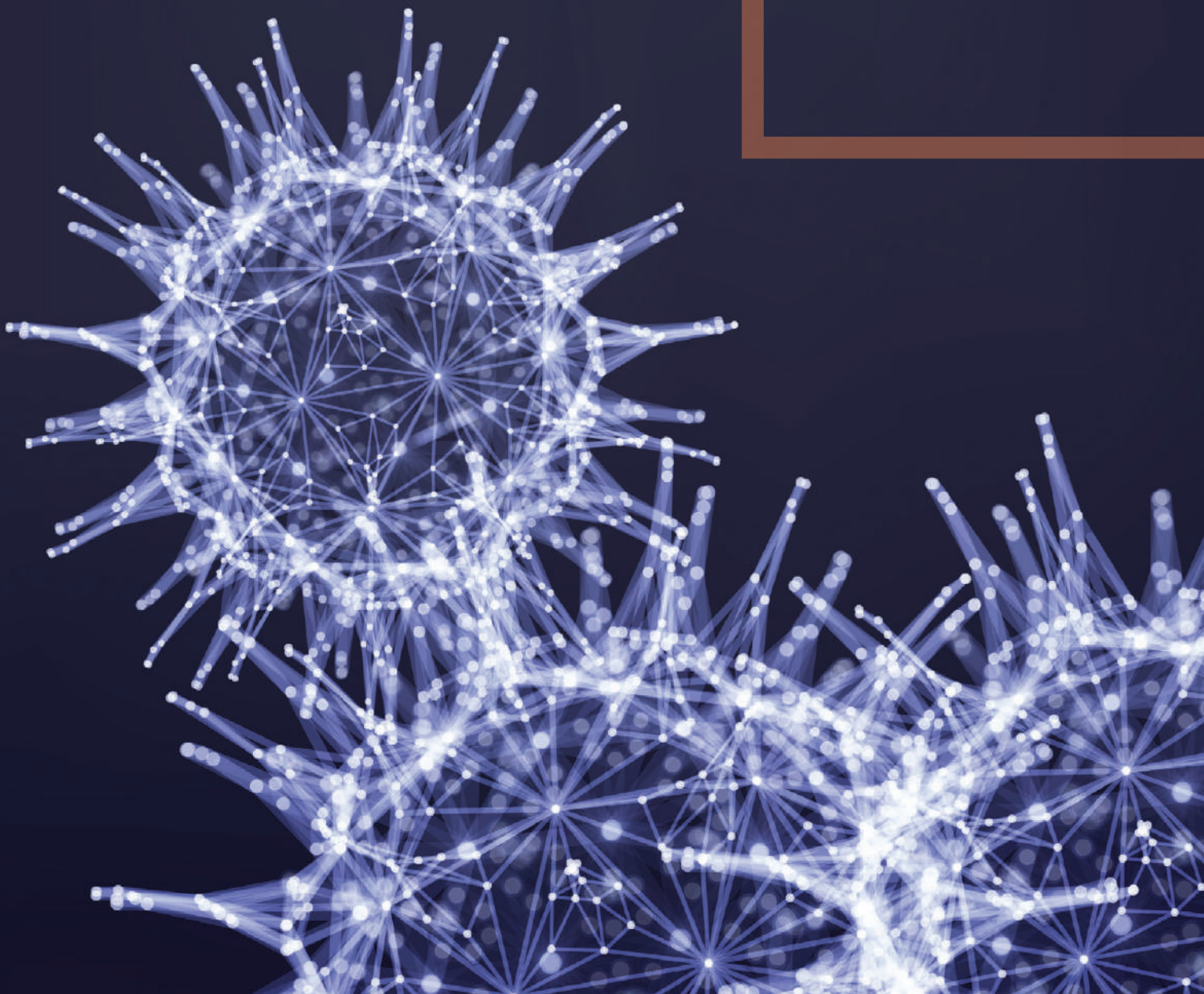
Abstracts of the Tenth International Workshop on HIV Persistence during Therapy
December 13 to 16, 2022
Miami, Florida, USA



Journal of Virus Eradication

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Contents

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Abstracts of the Tenth International Workshop on HIV Persistence during Therapy

Session 1: Basic Science of HIV Persistence

OP 1.1

Sequencing HIV: Significance and Impact

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Genetic characterization of HIV-1 proviruses isolated from CD4 + T cells of individuals on effective antiretroviral therapy has revealed that only 2–12% of persistent HIV-1 is genetically-intact and potentially replication-competent. Replication-competent proviruses are the main barrier to HIV-1 eradication as they contribute to viral rebound if therapy is interrupted.

Therefore, determining the source of latent replication-competent HIV-1 is important for identifying targets for future eradication strategies. Resting memory CD4 + T cells are a well-defined reservoir of latent HIV-1, however, several research groups have shown that this replication-competent HIV-1 is disproportionally distributed within different CD4 + T cell subsets. The different subsets of memory CD4 + T cells exhibit unique qualities that likely affect the genetic landscape of persistent HIV-1. In this presentation, we will explore the HIV-1 proviral landscape within CD4 + T cells subsets and identify potential cellular and viral mechanisms which contribute to the persistence of genetically-intact and defective HIV-1 proviruses within these subsets during therapy.

The genetic profile of plasma-derived HIV-1 RNA from viremic individuals during pre-therapy or after treatment interruption will also be discussed. Studies of the HIV-1 genetic landscape, during therapy or when therapy is stopped, inform our understanding of the cellular and viral mechanisms contributing to persistent HIV-1 which is critical for developing future curative strategies.

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OP 1.2 – 00157

HIV Silencing and Cell Survival Signatures of HIV-Infected CD4 T Cell Transcriptomes under Antiretroviral Therapy (ART)

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Background: Rare memory CD4 T cells harboring HIV under antiretroviral therapy (ART) represent an important barrier to HIV cure, but the infeasibility of isolating and characterizing these cells in their natural state has bred uncertainty about whether they possess distinctive attributes that HIV cure-directed therapies might exploit.

Methods: We developed a custom microfluidic process termed Focused Interrogation of cells by Nucleic acid Detection and Sequencing (FIND-Seq), which captures polyadenylated RNA and genomic DNA from millions of single cells within water-in-oil droplets and then sorts single-cell transcriptomes based solely on HIV DNA detection. Using peripheral blood cells from people with HIV who had initiated ART during chronic infection and had experienced >1 year of virologic suppression (n = 6), memory CD4 T cell transcriptomes were sorted by FIND-Seq into HIV DNA + and uninfected fractions and sequenced. Host cell transcriptomic profiles of HIV DNA + and uninfected memory CD4 T cells were compared by differential gene expression, pathways enrichment analysis, co-expression network analysis, and gene ontology.

Results: HIV DNA + memory CD4 T cells from ART-treated PWH showed inhibition of six transcriptomic pathways including death receptor signaling, necroptosis signaling, and Gα12/13 signaling. Gene co-expression network analysis revealed two small modules of genes associated with HIV DNA + cells. Gene ontology indicated significant enrichment of these clusters for factors related to the regulation of gene expression, RNA

processing, and certain cell state functions. Individual genes in these modules included HIV transcriptional activators that were lower in HIV DNA+ cells and HIV silencing factors affecting both transcriptional and post-transcriptional steps in HIV gene expression that were higher in HIV DNA+ cells. Remaining genes in these clusters not previously associated with HIV gene expression had roles in chromatin modification, RNA processing, and the survival and proliferation of CD4 T cells.

Conclusions: Whole transcriptome sequencing of HIV DNA+ memory CD4 T cells under ART in their natural state reveals these cells as a highly selected population whose host gene transcriptomic patterns favor HIV silencing, cell survival, and cell proliferation, with important implications for the development of HIV cure strategies.

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OP 1.3 – 00017

The fraction of cells with unspliced HIV RNA is not associated with plasma viremia

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Background: Untreated viremic controllers (VC) have low levels of plasma HIV RNA compared to untreated non-controllers (NC). We asked if the different levels of viremia between VC and NC were better explained by the fraction of infected peripheral blood mononuclear cells (PBMC) containing unspliced HIV RNA (usRNA) or by the number of copies of usRNA in single infected cells.

Methods: PBMC were obtained from donors with chronic HIV infection who were untreated VC (n = 8; plasma HIV RNA 60–2000 copies/mL), untreated NC (n = 7; 5700–275 000 copies/mL), or NC on ART (n = 5; <50 copies/mL). We applied cell-associated HIV RNA and DNA single-genome sequencing (CARD-SGS) to estimate the total number of infected cells/million PBMC,

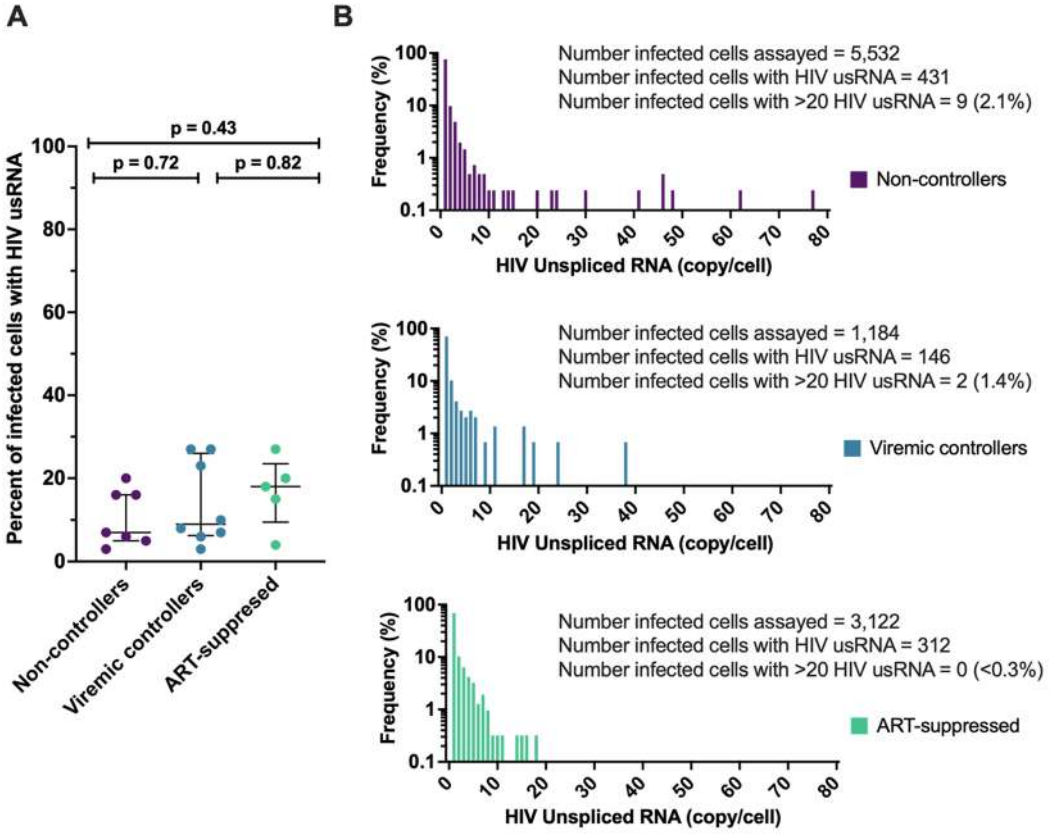

Figure 1. No difference in the fraction of infected cells with HIV usRNA in non-controllers, viremic controllers, and donors on ART. (**A**) The fraction of infected cells with HIV usRNA measured by CARD-SGS (ordinary one-way ANOVA with Tukey's multiple comparison test). (**B**) Distribution of the number of HIV usRNA copies per cell in each group.

Figure 1 (abstract: OP 1.3–00017) AAC Figure.

the fraction of infected cells containing usRNA, and levels of usRNA in single infected cells (N = 9557).

Results: The number of infected cells was significantly lower in VC (median 32 HIV DNA/million) compared to NC (1092/million; $p < 0.01$) and NC on ART (117/million; $p < 0.01$). The number of cells with HIV usRNA/million PBMC was significantly lower in VC (median 2.3/million) compared to NC (89.4/million; $p < 0.01$) and NC on ART (45.8/million; $p < 0.01$). Both were positively correlated with the level of viremia in the untreated donors (Spearman = 0.77; $p = 0.001$). By contrast, the fraction of infected cells with usRNA did not differ across the 3 groups (median 9% VC, 7% NC, and 18% NC on ART; $p = 0.46$; Figure 1A) and was not associated with plasma viremia (Spearman = −0.32; $p = 0.18$). Single infected cells with >20 molecules of usRNA were detected in 6/7 donors in the NC group, 2/9 donors in the VC group ($p = 0.04$) and were not detected in NC on ART (0/5 donors; Figure 1B).

Conclusions: These data reveal that levels of plasma viremia are determined by the total number of infected cells and the number of rare cells with high levels of HIV usRNA but not by differences in the fraction of infected cells with HIV usRNA. The finding that ~80–95% of infected cells in individuals with chronic HIV infection do not contain HIV usRNA, independent of levels of plasma viremia or ART status, implies that viremic control is not from a block to proviral expression, but rather by a block of viral spread to other cells.

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OP 1.4 – 00053

Definitive evidence of a persistent HIV reservoir in human brain myeloid cells despite ART

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Background: Long-lived and self-renewing tissue myeloid cells, such as brain microglia may harbor persistent, replication-competent HIV, and serve as reservoir, igniting rebound viremia following cessation of ART. Here, we isolated human brain myeloid cells (BrMCs) from rapid autopsy donations of people with HIV (PWH) under suppressive antiretroviral (ART), and assessed replication-competent HIV recovered from BrMC.

Methods: Cortex and basal ganglia regions from the brain of PWH (n = 4) were obtained through rapid autopsy of participants in the "Last Gift" cohort. CD3+ CNS T cells and CD11b+ BrMCs were isolated with a careful sequential protocol. Replication competent HIV reservoirs were measured by the quantitative viral outgrowth assay. BrMCs and CNS T cells from HIV-seronegative donors were included as controls (n = 4).

Results: Up to 1 × 106 viable BrMCs per gram of brain tissue (up to 60 grams in total) were isolated from PWH (n = 4), in which up to 95% BrMCs were TMEM119+ microglia. The levels of total and integrated HIV DNA were similar in BrMCs isolated from ART-suppressed (n = 2) and ART-interrupted PWH (n = 2, ART stopped < 3 weeks before the death). HIV largely remained silent in BrMCs in which the latent HIV was inducible by epigenetic regulators including histone deacetylation inhibitor SAHA and the methytransferase inhibitor CM272, but not the NF-κB agonist PEP005, suggesting difference in mechanisms controlling latency in these cells. Viral outgrowth was detected after induction and co-culture of BrMCs with PBMC PHA blasts. HIVLG29, a virus that was recovered in induced BrMC from one ART-suppressed donor productively infected both BrMCs and PBMC isolated from HIV-negative donors. Furthermore, HIVLG29 env sequence analysis after single genome amplification revealed its CNS origin and CCR5 tropism. Low CD4 usage was demonstrated, confirming that HIVLG29 is R5 microglia/macrophage tropic. Conversely, CNS T cells yielded no viral outgrowth, although 10–1000 × fewer T cells were obtained than BrMCs.

Conclusions: Our data demonstrate the first evidence for replication-competent HIV in isolated BrMCs, most probably microglia, from virally suppressed PWH, suggesting a true and main HIV reservoir within the CNS.

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OP 1.5 – 00046

P400/Tip60 chromatin remodeling complex in HIV transcription and latency establishment

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Background: Current HIV cure approaches, such as the block-and-lock and shock-and-kill strategies, depend on a complete understanding of the regulation of HIV transcription and the surrounding chromatin environment. There are ~320 chromatin regulatory factors (CRFs) that alter chromatin structure in a complex process, and characterization of chromatin regulators' roles in HIV transcription will potentiate development of multi-angled approaches towards HIV transcriptional regulation.

Methods: We performed a pooled RNAi screen utilizing shRNAs embedded in a microRNA backbone (shRNAmirs) to probe all human CRFs simultaneously in the J-Lat 10.6 HIV latency model. Following hit identification, we validated the role of EP400 complex (TIP60/NuA4 complex) in various latency models by a combination of knockdown, overexpression, and mutational studies. The binding of the EP400 complex on HIV DNA were analyzed by native ChIP-PCR, and ChIP-seq and RNA-seq were used to characterize genome-wide EP400 complex transcriptional regulation.

Results: EP400 was identified in our pooled RNAi screen as a key regulator of HIV latency. RNAi of EP400 and the other

components of the EP400 complex (DMAP1, BRD8, KAT5, EPC1, TRRAP) led to significant HIV reactivation, suggesting this complex is involved in maintaining transcriptional silencing of HIV provirus. RNA-seq analysis revealed that HIV transcripts were the highest and most significantly upregulated genes upon EP400 and DMAP1 RNAi. A dual colour HIV vector (HIVGKO) was used to segregate active and latent HIV infected cells. A significantly larger proportion of integrated cells were found to be actively transcribing HIV, while a smaller proportion was latent upon EP400 complex RNAi, suggesting that EP400 complex regulates HIV latency establishment. EP400 complex associates with the proviral DNA with a peak at the HIV 5′LTR, suggesting this complex may regulate HIV transcription directly. Interestingly, there was a high degree of concordance between RNAPII, EP400 and DMAP1 binding genome-wide by ChIP-seq, suggesting this complex might directly associate with and regulate RNAPII. This result is supported by Co-IP studies revealing an association of EP400 complex with RNAPII.

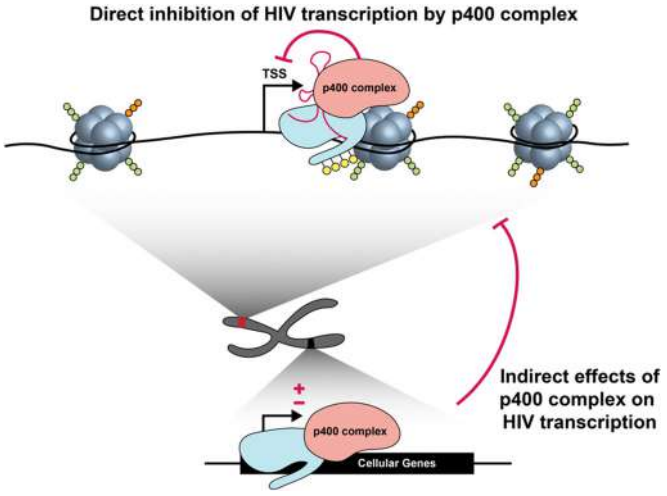

Figure. A model for p400 anti-HIV activity

Conclusions: EP400 complex plays a previously unappreciated important role in silencing HIV transcription, it appears to act directly at the HIV LTR to negatively regulate HIV transcription.

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OP 1.6 – 00138

Role of UHRF1 in HIV-1 transcriptional repression through epigenetic and non-epigenetic mechanisms

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Background: Studying the molecular mechanisms that drive HIV-1 into latency is critical for targeted HIV cure efforts aiming at either reactivating or blocking viral expression. We have recently reported the involvement of the cellular epigenetic integrator UHRF1 (Ubiquitin-like with PHD and RING finger domain 1) in the epigenetic control of HIV-1 latency (Verdikt et al., 2022, eBioMedicine). Here, we further characterized the epigenetic mechanisms of UHRF1-mediated silencing of HIV-1 and we demonstrated a non-epigenetic function of UHRF1 in HIV-1 transcription repression.

Methods: Sodium bisulfite sequencing, ChIP-qPCR, RNA interference, p24 ELISA, purification of primary cells from HIV + patient blood, co-immunoprecipitation, mass spectrometry.

Results: Using T cell line and primary T cell models for HIV-1 latency, we showed that UHRF1 was redundantly recruited to the latent HIV-1 promoter through different binding modalities where DNA methylation was either non-essential, essential or enhancing UHRF1 binding. Genetic or pharmacological UHRF1 downregulation induced an increase in HIV-1 gene expression accompanied by decreased recruitment of DNMT1, G9a and HDAC1 to the HIV-1 promoter. Moreover, UHRF1 acted by a similar epigenetic mechanism in latently-infected cells from myeloid origin. We also showed that UHRF1 repressed HIV-1 in absence and presence of Tat, independently of 5′LTR DNA methylation. UHRF1 possesses an E3-ubiquitin ligase activity and we showed that deletion of the C-terminal end of UHRF1 containing its catalytic RING domain strongly reduced UHRF1 ability to repress HIV-1 transcription. In addition, co-immunoprecipitation assays demonstrated the interaction of UHRF1 with Tat. However, UHRF1 overexpression had no effect on Tat stability contrarily to what has been previously reported by others (Liang et al., 2021, mBio). Finally, we will evaluate by mass spectrometry the cellular proteins associated with the UHRF1 catalytic RING domain and data will be presented.

Conclusions: We demonstrated a role of UHRF1 in the epigenetic repression of the latent HIV-1 in both T-lymphoid and myeloid reservoirs. Our results also suggested the involvement of the ubiquitin ligase activity of UHRF1 in the non-epigenetic mechanism(s) of UHRF1-mediated HIV-1 repression. Our findings highlight the ability of UHRF1 to repress HIV-1 transcription through multiple mechanisms and therefore reinforce its relevance as an attractive therapeutic target for anti-HIV cure strategies.

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OP 1.7 – 00194

Potent latency reversal enables in-depth transcriptomic analysis of the translation-competent HIV-1 reservoir

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Background: Extensive characterization of the translation-competent reservoir has been hampered by the limited capacity of current latency reversing agents (LRAs) at inducing HIV reactivation in vitro. Here, we tested the capacity of a Tat mimetic (Tat #1) in combination with other LRAs such as panobinostat (PNB) or PMA to induce HIV reactivation in CD4 T cells from ART-suppressed individuals.

Methods: CD4 T cells from ART-treated individuals were stimulated for 24 h with Tat #1/PNB or Tat #1/PMA. PMA/ionomycin (PMA/i) stimulation was used as a positive control. The frequency of cells expressing p24 was assessed by HIV-Flow. Following Tat #1 treatment, single-cell RNA-sequencing (scRNA-seq) on sorted p24-/p24+ cells from 7 ART-treated individuals was used to study the transcriptome of the inducible reservoir. Transcriptomic differences between p24+ and p24- cells were validated at the protein level using flow cytometry.

Results: Tat #1/PNB induced HIV reactivation in a larger fraction of CD4 T cells than PMA/i ($n = 22$, $p < 0.00001$, fold increase = 3.9). Interestingly, when CD4 T cells were stimulated with Tat #1/PMA, a fold increase of 9.5 times was observed in the frequency of p24+ cells compared to PMA/i ($n = 6$, $p = 0.03$). scRNA-seq analyses showed that Tat #1 does not modify the cellular transcriptome of CD4 T cells, allowing to study the transcriptomic features of the inducible reservoir in its near-native state. Following reactivation with Tat #1, p24+ cells significantly expressed higher levels of a long non-coding RNA, *SOD1P3*, *CCL5* and *GZMA*, while expressing lower levels of *ATG10* and *IL7R* when compared to p24- cells. Flow cytometry analyses confirmed that p24+ cells expressed higher levels of CCL5 and GZMA proteins while expressing lower levels of IL7R and GZMB proteins compared to p24- cells.

Conclusions: We report combinations of LRAs that induce latency reversal in a higher proportion of latently infected cells compared to PMA/i, suggesting that PMA/i only reactivates a small fraction of the translation-competent reservoir. Moreover, using single-cell RNA sequencing, we showed that p24+ cells display a distinct transcriptional signature compared to non-infected cells and we validated the transcriptomic hits at the protein level.

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OP 1.8 – 00204

Romidepsin in combination with the BCL-2 antagonist venetoclax synergistically reduce the size of the HIV reservoir

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Background: Latency reversing agents (LRAs) can successfully induce HIV transcription in people living with HIV (PLWH) on antiretroviral therapy (ART), however, a reduction in the HIV reservoir has not been observed. One potential explanation is that latently infected cells over express pro-survival proteins such as BCL-2. Given that the HIV proteins can induce apoptosis in CD4+ T-cells, we hypothesised that the combination of a potent LRA with the BCL-2 antagonist, venetoclax, will enhance elimination of latently infected cells.

Methods: Total CD4+ T-cells were isolated from blood collected by leukapheresis from PLWH on ART ($n = 6$). Venetoclax (5 nM, 10 nM and 100 nM) was added for 24-hours, followed by treatment with the LRA romidepsin (20 nM) for 4-hours. Cells were washed and cultured for an additional 20-hours. Total integrated HIV DNA, intact and defective DNA, cell-associated unspliced and multiply spliced HIV RNA were quantified by qPCR. Changes in T-cell subsets were quantified using flow cytometry.

Results: Venetoclax alone induced dose dependent cell toxicity in total CD4+ T-cells however, viability was >80% with all concentrations. Naive and effector memory CD4+ T-cells compared to other T-cell subsets were more susceptible to cell death with venetoclax. The combination of romidepsin together with venetoclax at 5 nM, 10 nM and 100 nM, significantly reduced integrated HIV DNA ($p < 0.0005$, $p < 0.005$ and $p < 0.05$, respectively), and this combination was synergistic compared to either drug alone (Bliss independence scores for each concentration of venetoclax was of 0.36, 0.59 and 0.20 respectively). Using the intact proviral DNA assay in 3 donors, we observed a reduction in intact HIV DNA in 3 of 3 donors with 10 nM venetoclax and in 2 of 3 donors with 5 nM and 10 nM venetoclax with romidepsin. This combination also induced a significant increase in unspliced HIV RNA ($p = 0.03$). Unexpectedly, venetoclax alone induced expression of both unspliced and multiply spliced HIV RNA.

Conclusions: Reductions in integrated HIV DNA in CD4+ T-cells from PLWH on ART *ex vivo* was enhanced using the pro-apoptotic drug venetoclax together with the LRA romidepsin. Venetoclax may also directly activate HIV transcription. Romidepsin together with venetoclax is an effective combination in reducing the HIV reservoir and should be further explored in additional pre-clinical models.

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OP 1.9 – 00042

Measuring the impact of early 3BNC117 intervention at ART initiation on the productive reservoir in a cohort of diverse viral subtypes: results from the VIP-SPOT assay in the eCLEAR trial

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Background: The persistent reservoir of latently HIV-infected cells, established soon after viral acquisition, is the reason why antiretroviral therapy (ART) is unable to cure HIV. The eCLEAR study has demonstrated that broadly neutralizing antibodies, given at the time of ART initiation can induce a period of post-antiretroviral remission. We explore how this intervention has

impacted measures of the productive HIV reservoir using the VIP-SPOT assay.

Methods: eCLEAR trial (NCT03041012) is a phase 1b/2a, open-label, multicenter, randomized controlled trial among people starting ART randomly allocated to one of 4 treatment groups: ART alone, ART + 3BNC117, ART + romidepsin, ART + 3BNC117 + romidepsin (3BNC117 on days 7 + 21 followed by romidepsin on days 10 + 17 + 24 after ART initiation). eCLEAR assessed whether administration of the bNAb 3BNC117, with or without the latency-reversing agent romidepsin, could potentially enhance clearance of HIV-infected cells. Cryopreserved PBMCs from 50/60 study participants were used to evaluate the productive viral reservoir 1 year after ART initiation using the novel VIP-SPOT assay. We compared raw VIP-SPOT data with plasma viremia, HIV viral clade from sequencing, proviral HIV DNA, and intact provirus measured by an IPDA-like duplexed ddPCR (3dPCR) assay.

Results: Before ART initiation, the frequency of HIV-antigen producing cells measured by VIP-SPOT strongly correlated positively with plasma viremia ($P = 0.001$), total and intact HIV DNA in peripheral CD4+ T cells ($P < 0.001$). No differences in the frequency of detection of HIV-Ag producing cells were observed between B and non-B HIV-1 subtypes.

One year after ART initiation, the productive reservoir was significantly reduced with a median decrease >1 log in all 4 groups ($P = 0.0025$, 0.0012, 0.014 and 0.0015 respectively) compared to baseline. The proportion of individuals whose productive reservoir reached undetectable values was similar between study groups, but a trend towards greater undetectability was observed in participants receiving 3BNC117, either alone or in combination with romidepsin, especially in individuals whose pre-ART plasma viruses were sensitive to 3BNC117 compared to those harbouring resistant viruses (47% vs. 33%).

Conclusions: The VIP-SPOT assay was useful for evaluating the impact of interventions on the productive reservoir regardless of HIV-1 subtype and evidenced the clearance of CD4+ cells capable of producing HIV-1 protein upon ART initiation.

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OP 1.10 – 00210

Clonal Dynamics within HIV-Infected CD4 T Cell Reservoirs after PD-1 Blockade under ART

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Background: Blockade of the programmed cell death protein-1 (PD-1) pathway can reverse HIV latency, potentiate virus-specific CD8 T-cell responses, and has been associated with favorable outcome in some cases of central nervous system (CNS) infection. The effects of PD-1 blockade on CNS reservoirs for HIV are under study in clinical trial NCT03239899. We evaluated HIV reservoirs in blood in participants from this trial.

Methods: People with HIV on ART and CD4 counts >350 cells/μL received one infusion of 200 mg pembrolizumab. PBMC were collected at baseline, week 3 and 24 post-infusion. CD4 T-cells were FACS-sorted into naïve, central/transitional memory, and effector memory (EM) subsets. HIV DNA was quantified by limiting dilution PCR of env and Sanger sequencing. HIV RNAs were quantified by ddPCR. Changes in gene expression patterns and T-cell receptor (TCR) repertoires were evaluated. Bulk ISA in CD4 T-cells was used to assess similarity and clonality of all IS within each person across timepoints. Intact HIV proviruses in CD4 T-cells were enumerated with intact proviral DNA assay.

Results: Pembrolizumab infusion and 24 weeks of follow-up were completed for six participants. A shift in distribution of HIV-infected cells to the EM subset and a reduced genetic diversity of HIV DNA sequences were observed post-infusion. These were associated with transcriptomic signatures of increased cell cycling, expansion of a small number of HIV-infected cell clones and increasing HIV transcriptional initiation at week 3 post-infusion. While expanded TCR clonotypes showed stable frequencies across timepoints, the diversity of the TCR repertoire in EM cells increased 3 weeks after treatment. In one participant, one clone expanded post-infusion to account for ~1% of all circulating CD4 T-cells. Significant changes in the IS population were observed after pembrolizumab in one participant with a large number of detected IS. The number of intact proviruses had no consistent change over time.

Conclusions: PD-1 blockade was associated with a shift to an EM phenotype, HIV latency reversal, changes in the IS landscape, and expansion of some HIV-infected cell clones. Further investigation of intact proviruses in T-cell clones following checkpoint inhibitor administration will help clarify the net effect of these agents in HIV cure strategies.

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PP 1.1 – 00009

TB-associated microenvironment promotes HIV latency in CD4+ T cells

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Background: HIV-infected individuals are frequently co-infected with Mycobacterium tuberculosis (Mtb)-the causative agent of tuberculosis (TB). The reduction of CD4 T-cells associated with HIV infection increase the risk of active TB. However, the effects of the TB-associated microenvironment on HIV infection and latency in CD4 T-cells is unclear.

Therapeutically aspirated pleural effusions from TB patients (TB-PE), can reflect the microenvironment found in human respiratory cavities impacted by Mtb infection. Therefore, we investigated the effects of TB-PE on HIV replication and latency within CD4 T-cells.

Methods: CD4 T-cells were isolated from 6 healthy-donors and infected with HIV_{NL4-3} in the presence or absence of TB-PE. Viral entry was assessed through the HIV-Vpr-BlaM system, reverse transcription was quantified by qPCR, and the percentage of HIV-infection measured by p24 immunostaining. For HIV latency studies, CD4 T-cells from 4 healthy donors were infected with a dual-fluorescent reporter HIV. Cells latently infected with this reporter virus express the near-infrared fluorescent protein (iRFP) while cells undergoing a productive infection express both iRFP and the green fluorescent protein (GFP). The proportion of latently HIV-infected cells, in the presence or absence of TB-PE, was quantified by flow cytometry. To test whether TB-PE affects HIV latency reversal, CD4 J-Lat cells were exposed to PMA with or without TB-PE and the expression of GFP measured by flow cytometry.

Results: We observed that the reverse transcription, and percentage of HIV infection in CD4 T-cells decreased in the presence of TB-PE ($p < 0.05$). However, this was not due to differences in the viral entry. We also observed that the incubation with TB-PE significatively increased the proportion of latently HIV-infected cells ($p < 0.05$). In addition, PMA-induced latency reversal of CD4 T-cells was significatively reduced with TB-PE treatment ($p < 0.05$).

Conclusions: Our results indicate that the presence of TB-PE negatively impacts viral replication, induces HIV latency, and decreases latency reversal in CD4 T-cells. Importantly, our study suggests that TB-associated microenvironments may contribute to HIV persistence and the viral reservoir and pose a challenge to HIV curative strategies, such as shock and kill, which rely on latency reversal in co-infected patients.

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PP 1.2 – 00011

Structural rearrangements in the nucleus localize latent HIV proviruses to a perinucleolar zone supportive of early transcription reactivation

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Background: HIV proviruses enter latency when effector cells transition to memory, a process associated with shutdown of the transcriptional machinery due to disruption of the 7SK RNP complex containing P-TEFb. We discovered recently that proviral localization is affected by rearrangements in the nuclear landscape during effector cell transition to quiescence.

Methods: To visualize the spatiotemporal dynamics of HIV DNA from acute infection, through effector cell transition to quiescence when the proviruses become latent, and subsequently after cell activation, we developed a novel CasFISH technique to label HIV DNA sequences in the LTR.

Results: In Th17 cells acutely infected with a single-round HIV-1 reporter virus, capsid-associated HIV DNA was found initially at the nuclear envelope together with cleavage and polyadenylation specificity factor 6 (CPSF6). Following nuclear entry, capsid dissociated in the nuclear periphery and only integrated HIV DNA/CPSF6 complexes were detected. During the cellular transition to quiescence there was a dramatic increase in histone methylation, compaction of the nuclear DNA, loss of CPSF6, and the establishment of viral latency. During this process large scale chromatin rearrangements led to the accumulation of proviral DNA in the perinucleolar region. Proviral perinucleolar localization required integration and was blocked by Raltegravir.

Following reactivation of the latently infected cells through their T-cell receptors, nascent viral mRNA transcripts can be detected emanating from proviral transcription sites localized in the perinuclear zones. The viral trans-activator Tat and its proviral reactivation regulatory partners, P-TEFb and 7SK snRNA rapidly accumulated in large interchromatin granular clusters which assembled near the proviruses in the perinucleolar zone.

As cellular reactivation, a process associated with nucleolar expansion progressed, nuclear rearrangements led to a shift of the proviral DNA further away from the nucleolus. Similarly, the majority of proviruses in the latently infected memory T-cells from well-suppressed patients also accumulated in the perinucleolar zone and showed identical patterns of RNA transcription after cell activation.

Conclusions: Thus, specific global changes in the nuclear architecture relocates latent proviruses in the perinucleolar zones of resting T cells, a region that is highly supportive of early proviral transcription reactivation after cell activation.

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PP 1.3 – 00022

Signaling pathways that activate P-TEFb to reverse HIV latency in CD4+ T cells

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Background: Emergence of HIV from latency requires an autofeedback mechanism initiated by the viral trans-activator Tat, which functions to recruit the host transcription elongation factor P-TEFb to proviral HIV. In primary T cells generation of active P-TEFb, a heterodimer of CDK9 kinase and cyclin T1, occurs in a sequence of molecular events that depend upon T-cell activation signals. This research was aimed at identifying specific primary T-cell signaling pathways that mediate the biogenesis of P-TEFb to stimulate latent HIV reactivation.

Methods: Using a well-described primary Th17 cell model of HIV latency alongside healthy donor-derived memory CD4+ T cells, we employed intracellular immunofluorescence flow cytometry as well as single-cell and bulk RNA-seq studies to define T-cell receptor (TCR) pathways that regulate the generation of transcriptionally active P-TEFb, defined as the coordinate expression of cyclin T1 and phospho-Ser175 CDK9.

Results: Protein kinase C (PKC) agonists, namely ingenol, prostratin and bryostatin stimulated active P-TEFb expression and reactivated latent HIV even without intracellular calcium mobilization with an ionophore. However, while ingenol, prostratin and TCR-mobilized diacylglycerol were found to

signal through MAP kinases ERK1/2 rather than PKC to effect the reactivation of both P-TEFb and latent HIV, bryostatin utilized both PKC-θ and MAPK ERK1/2 pathways to effect these processes. Single-cell and bulk RNA-seq analyses revealed that of the four known isoforms of the Ras guanine nucleotide exchange factor RasGRP, RasGRP1 is by far the predominantly expressed diacylglycerol-dependent isoform in CD4+ T cells. RasGRP1 should therefore mediate the activation of ERK1/2 via Ras-Raf signaling upon TCR co-stimulation or PKC agonist challenge. By contrast, expression of the diacylglycerol-independent isoform RasGRP2 was restricted to resting T cells with both its transcripts and protein exhibiting a short-lived half-life in TCR-activated cells. Combined inhibition of the PI3K-mTORC2-AKT-mTORC1 pathway and the ERK1/2 activator MEK prior to TCR co-stimulation abrogated active P-TEFb expression and substantially suppressed latent HIV reactivation.

Conclusions: The RasGRP1-Ras-Raf-MEK-ERK1/2 and PI3K-mTORC2-AKT-mTORC1 pathways complement one another in stimulating the translation of pre-existing cyclin T1 mRNA leading to heterodimer assembly of P-TEFb. Therefore, selectively targeting RasGRP1 with diacylglycerol mimics that weakly bind PKC enzymes would be a potentially optimal strategy for HIV latency reversal.

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PP 1.4 – 00037

Histone decrotonylation uniquely regulates HIV-1 transcription and can be modulated to control HIV-1 latency

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Background: Latent reservoirs in people living with HIV-1 are a substantial roadblock to obtaining a cure. Methods to control latent HIV-1 have targeted a variety of signaling pathways including histone PTMs. Histone crotonylation is a recently discovered epigenetic marker present during active gene transcription of HIV-1 while the inhibition of histone crotonylation reduces HIV transcription. *We hypothesize that decrotonylation has a unique role in regulating HIV latency.*

Methods: HIV-1 reporter cell lines (TZM-bl and 2D10) and primary T cell model were used to examine the effects of histone crotonylation on HIV latency. TZM-bl cells were used to determine the impact of p300 and HDAC3 and their mutants on Tat transactivation of HIV transcription. Histone crotonylation and acetylation levels were determined through western blotting of histone extracts. Transcription of HIV was measured by RT-qPCR using the HIV-1 long LTR primers/probe.

Results: We discovered that Tat-driven HIV transcription is suppressed by the decrotonylase activity but not the deacetylase activity of HDAC3, as seen by inhibition of Tat transactivation in cells expressing either wildtype HDAC3 or decrotonylase only mutants of HDAC3. The importance of histone crotonylation is further supported by enhanced Tat transactivation in cells expressing crotonyltransferase only p300, similar to wildtype p300. We have shown that most HDAC inhibitors have dual activities also acting as histone decrotonylase (HDCR) inhibitors. Interestingly, a subset of HDAC inhibitors containing benzamide zinc binding groups display specificity for inhibiting HDCR activity over HDAC activity. We have further modified a commercially available HDAC3 specific inhibitor, RGFP966, to be a potent HDCR inhibitor with a more robust activity to disrupt HIV latency.

Conclusions: We conclude that histone crotonylation regulates HIV transcription independent of histone acetylation. The currently available HDAC inhibitors are non-specific towards deacetylation. Notably, the use of more specific HDCR inhibitors may result in stronger latency reversal activity while minimizing off target effects. The discovery of such a compound with selective HDCR inhibition over HDAC inhibition provides a useful tool for dissecting the molecular basis of these two similar but distinct PTMs during HIV latency, leading to the design of more specific small molecules to control stable HIV reservoirs.

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PP 1.5 – 00040

Mapping of genetic interaction networks identifies a nucleosomal modification complex for silencing HIV

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Background: Recent identification of numerous host inhibitors for HIV latency reactivation reflects the complex molecular mechanisms that contribute to the extraordinary stability of latent HIV reservoir. But the topology of the underlying networks is largely unknown.

Methods: We developed Reiterative Enrichment and Authentication of CRISPRi Targets for Synergies (REACTS) that has allowed unambiguous large-scale probing of synergistic effects in human cells.

Results: Using sgRNAs targeting 7 known host inhibitors as queries, we identified 30 synergies among 10 host inhibitors, including 3 new inhibitors BCL7C, KANSL2, and SIRT2. Their overexpression reduced spontaneous and PMA-induced HIV reactivation in J-Lat A2 cells, a Jurkat-based HIV latency model, as well as in CD4 T cells from people living with HIV. Mechanistically, BCL7C and KANSL2 form a complex to increase histone acetylation and Brd4S on HIV promoter.

Conclusions: Our study has established a pipeline for finding new synergistic host inhibitors toward a complete understanding of the molecular mechanisms of HIV latency, providing potential new targets for Block-and-Lock intervention.

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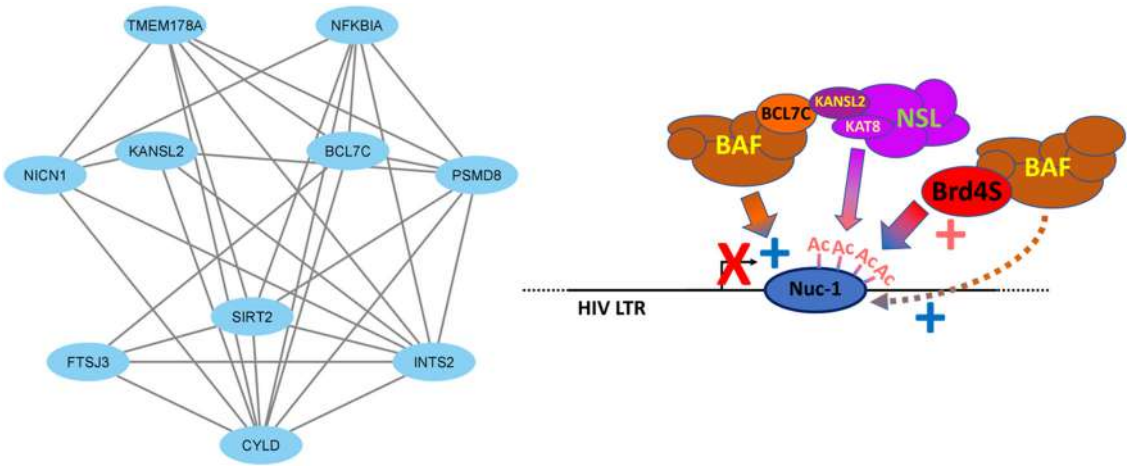

Figure 1 (abstract: PP 1.5–00040) Mapping of genetic interaction networks

PP 1.6 – 00047

LAIR-1 is a negative regulator of SIV-specific CD8 T cells during chronic SIV infection

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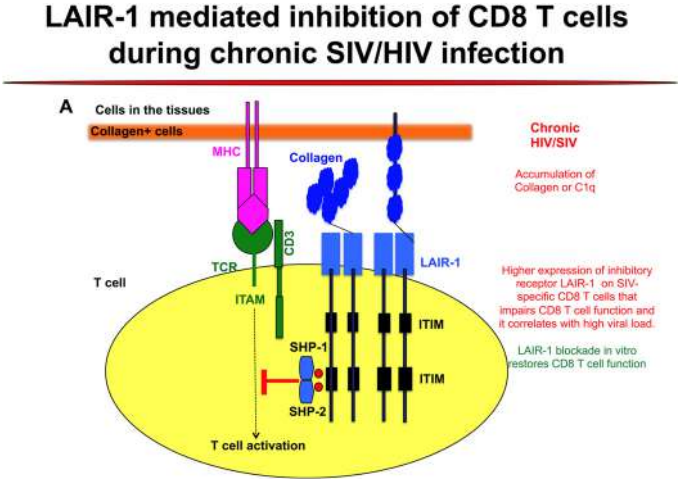
Background: Human Immunodeficiency virus (HIV)-1- specific CD8 T cells play an important role in controlling HIV infection. However, during chronic HIV infection, HIV-specific CD8 T cells undergo functional exhaustion, lose effector function, and fail to inhibit viral replication. Leukocyte-associated Ig-like receptor-1 (LAIR-1), a 32 kDa transmembrane glycoprotein, is a surface molecule expressed on human peripheral blood mononuclear leukocytes that function as an inhibitory receptor. However, the role of LAIR-1 on CD8 T cells during SIV/HIV infection remains to be fully elucidated.

Methods: A total of 14 macaques were infected with simian immunodeficiency virus (SIVmac251) and studied the temporal dynamics of LAIR-1+ SIV-specific CD8 T cells for their phenotypic and functional profiles using multicolor flow cytometry. We performed RNA sequencing to study the transcriptomic profile of LAIR-1+ CD8 T cells and performed immunohistochemistry IHC to stain for LAIR-1 in LN tissues during chronic SIV.

Results: Here, we report on the expression of LAIR-1 on virus-specific CD8+ T cells during chronic SIV infection and the effect of LAIR-1 blockade on the proliferation and cytokine function of these cells. LAIR-1 expression was found to be low on naive CD8+ T cells and increased on total and SIV-specific effector memory CD8+ T cells during chronic infection. In addition, the level of LAIR-1 expression in the lymph node during chronic infection was higher compared to naïve animals. Transcriptionally, these LAIR-1+ CD8 T cells exhibited a unique transcriptome characterized by with heightened type I IFN-signaling coupled with reduced TCR signaling, cell cycling and cell metabolism pathways. Importantly the expression of LAIR-1 on SIV-specific effector memory CD8 T cells correlated directly with the SIV viral RNA levels in plasma. Indeed, In vitro blockade of LAIR-1 using LAIR-1-Fc/anti-LAIR-1 antibody resulted in enhanced proliferation of SIV-specific CD8 T cells with cytotoxic function.



Conclusions: These results serve as a foundation for future in vivo trials of the use of LAIR-1 blockade to potentially enhance and/or restore antiviral SIV-specific CD8 T cells, especially in secondary lymphoid tissues which may be important for the HIV cure strategy.

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PP 1.7 – 00051

Continuous decline of intact proviral DNA after two decades of antiretroviral therapy

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Background: Implementation of antiretroviral therapy (ART) suppresses viral replication but does not eliminate the persistent HIV DNA reservoir in CD4$^+$ T cells. This reservoir contains predominantly defective proviruses, shielding the smaller intact proviral DNA reservoir that forms the major obstacle to HIV cure. To gain insight into the size and dynamics of this (intact) proviral DNA reservoir during treatment, we conducted a longitudinal study in 9 individuals who initiated ART over 20 years ago.

Methods: PBMCs were obtained before ART initiation, 1, 10 and 20 years after treatment start. DNA isolated from PBMCs and sorted T cell subsets (naïve, central-memory, transitional-memory, effector-memory, and effector T cells) at 10 and 20 years after ART was analysed using the intact proviral DNA assay (IPDA). This allowed quantification of defective and intact proviral DNA. The viral env region obtained from DNA samples and pre-therapy plasma viral RNA was deep-sequenced using MiSeq. Viral evolution was investigated using phylogenetic analyses.

Results: A continuous decline of intact proviral DNA was observed during 20 years of therapy, whereas a stabilization in the decline of the defective proviral DNA was observed (figure). Within the last decade, intact proviral DNA showed a flattening decline in both PBMC and T-cell subsets (figure). At 20 years after ART initiation, memory T-cell subsets contained the largest fraction of proviral DNA compared to the naïve subsets and PBMCs. The same trends were observed for 10 years after ART initiation. Phylogenetic analysis and root-to-tip distance analyses did not show signs of evolution over time.

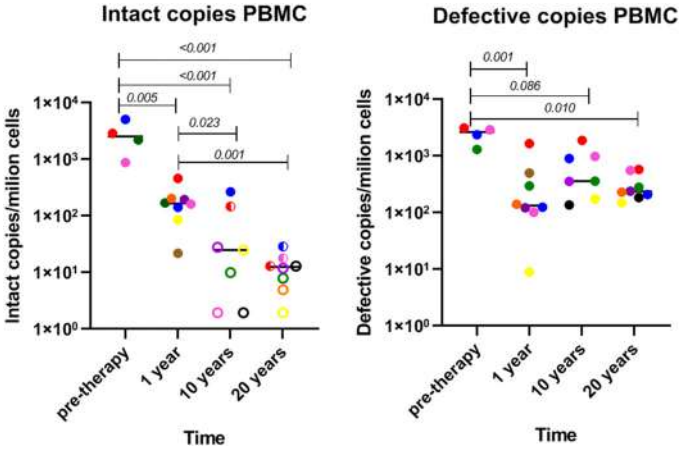

Figure. The dynamics of proviral DNA in the PBMC fraction.

Conclusions: The size of the intact proviral reservoir significantly declined during 20 years of ART. The proviral DNA could be a source of ongoing viral production. Consequently, the continuous decline within the intact proviral reservoir could be caused by virus-induced or immune-mediated cell killing. The lack of evidence of ongoing viral replication argues for persistence of the (defective) proviral reservoir via clonal/homeostatic expansion. Altogether, this study provides insights on the dynamics of the replication-competent viral reservoir during prolonged ART.

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PP 1.8 – 00054

Integrative epigenomic and transcriptomic analysis reveals distinct regulations of mRNAs and lncRNAs in active versus latent HIV-1 infection of T cells

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Background: Persistence of HIV-1 latently-infected T-lymphocytes under prolong cART is a major roadblock to complete viral remission and cure. Although regulations of viral transcription in latently-infected cells have been extensively studied, a comprehensive understanding of the cellular microenvironment that is conducive to establishment and maintenance of HIV-1 latency is also necessary for eliminating these viral reservoirs. We have undertaken an integrative and comparative analysis of the protein-coding and long non-coding RNA (lncRNA) transcriptomes and their regulations by epigenomic reorganization in active and latent HIV-1 infection of T cells.

Methods: We have used an in vitro model of HIV-1 latency in SupT1 cells, a lymphoblastic T cell line. We infected SupT1 cells with a single-round, dual-reporter virus, HIVGKO, which encompasses two distinct fluorescent readouts: one for viral infection and provirus integration (mKO2, under a constitutive cellular promoter EF1a) and another to indicate active viral transcription (codon-switched GFP under viral LTR promoter). Based on the reporter readouts, we flow-sorted the active- (mKO2 + GFP +) and latently- (mKO2 + GFP-) infected cells and then extracted genomic DNA and total cellular RNA from the flow-sorted cells for processing with ATAC-seq and RNA-seq, respectively.

Results: The ATAC-seq revealed a divergent regulation of chromatin reorganization between active- and latently-infected SupT1 cells compared to uninfected ones. Latent HIV-1 infection led to significant repression whereas active infection increased overall accessibility of the host chromatin. Furthermore, we observed a greater impact of chromatin reorganization on the transcription profiles of both mRNAs and lncRNAs in latently-infected cells that affected cellular pathways such as RNA metabolism, transcription and cell cycle. Integration of the ATAC-seq and RNA-seq data also revealed a divergent pattern of regulation by transcription factors. Additionally, we identified several new lncRNAs such as RP11-1C8.5 with potential roles in HIV-1 replication and latency.

Conclusions: The multi-omics analysis demonstrated that establishment and maintenance of HIV-1 latency in T cells require a significantly broader alteration in host cell epigenome and transcriptome than active infection and revealed new avenues for modulating these reservoir cells.

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PP 1.9 – 00067

Longitudinal quantification of HIV proviral DNA and host APOBEC3G/-F mRNA expression in cellular subsets that are targeted by HIV-1

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Background: The HIV reservoir is heterogenous in cellular and viral genetic composition, dominated by defective sequences with frequent APOBEC3G (A3G) and APOBEC3F (A3F) mediated hypermutations. The evolution of the reservoir has not been fully characterized and the cellular sources of A3G/-F hypermutations and the impact of antiretroviral therapy (ART) initiation on viral reservoirs within diverse immune cells have not been investigated. This study longitudinally quantified HIV proviral DNA and A3G/-F expression in immune cell subsets.

Methods: Five women with pre-infection samples were identified with hyperacute infection in Durban, South Africa. Participants initiated ART at a median of 783 days post infection. HIV proviral copy numbers and A3G and A3F mRNA levels were measured using ddPCR in CD4 + T-cell subsets (central memory (CM), effector memory (EM), transitional memory (TM), naïve (N)) and monocytes. Samples were analysed pre-infection, and at 1, 6 and 12 months post infection and similar timepoints post-ART.

Results: At 12 months post-infection, CM and EM T-cells harboured the highest levels of proviral DNA (~6300 copies/million cells), with approximately 10-fold lower levels in monocytes and naïve T-cells. Proviral DNA did not decrease significantly in any cell subset at 12 months post-ART. Before infection, A3G and A3F expression levels were similar across all cell subsets; with A3G expression higher than A3F for all subsets ($p = 0.0012$). Upon infection, both A3G and A3F levels were downregulated for all subsets ($p = < 0.0001$). After 1 year of treatment, A3G ($p = 0.0016$) and A3F ($p = 0.0058$) levels recovered but remained significantly below pre-infection levels.

Conclusions: CM and EM harboured the highest HIV proviral DNA levels but proviral DNA was also detectable in monocytes and naïve cells at all time points. There was no significant change in HIV proviral load in any cell subset at 12 months post-ART. A3G/-3F expression are downregulated after HIV infection but expression recovered following ART initiation. This study highlights that diverse immune cells stably harbour the HIV reservoir, with little impact of ART. APOBEC3 proteins are downregulated following infection and understanding the role these proteins play in shaping the proviral HIV reservoir may allow us to harness the innate immune system as a cure strategy.

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PP 1.10 – 00069

Isotretinoin enhances IL-15 mediated HIV latency reversal and reduces the inducible latent reservoir

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Background: Finding novel therapeutic strategies that reactivate latent HIV, enhance immune effector functions, and sensitize reactivated cells to apoptosis could improve the killing and elimination of the latent reservoirs. Among the clinically relevant latency reversing agents (LRA) under investigation, IL-15 or its superagonist N-803 have been shown to reactivate latent HIV *ex vivo* and *in vivo*. However, its clinical benefit can be hindered by the transient and unsustained nature of cytokine signaling. We previously identified a small molecule, HODHBt, that potentiates the biological activity of IL-15 by increasing STAT5 phosphorylation and transcriptional activity leading to enhanced IL-15-mediated viral reactivation *ex vivo* in cells isolated from ART-suppressed participants. Furthermore, HODHBt also increases IL-15 mediated NK effector function. In this work we aim to identify and evaluate the LRA potential of FDA-approved HODHBt transcriptional analogues.

Methods: We used the Connectivity Map to identify FDA-approved compounds with transcriptional profiles similar to HODHBt. We confirmed their biological activity in different cell models, including a primary cell model of latency, CD4 T cells isolated from 10 ART-suppressed people living with HIV, and *in vitro* recalled HIV-specific CD8 T cells.

Results: We identified the FDA-approved retinoid derivative Isotretinoin as a transcriptional analogue of HODHBt. Isotretinoin enhanced the latency reversal activity of IL-15 1.5-fold and reduced the size of the inducible latent reservoir 6.5-fold measured as %p24 positive cells upon reactivation by flow cytometry in a primary cell model of latency. Mechanistically, Isotretinoin promoted the activation of P38 leading to enhanced viral reactivation and increased expression of the pro-apoptotic protein NOXA measured by western blot, sensitizing HIV infected cells to cell death. Finally, Isotretinoin enhanced the killing capacity of HIV-specific CD8 T cells.

Conclusions: We have identified novel effects of Isotretinoin including the enhancement of IL-15-mediated latency reversal, depletion of the latent reservoir *in vitro*, and increased killing capacity of HIV-specific CD8 T cells. While further studies are warranted to evaluate the potential of this FDA-approved compound to reduce latent reservoirs *in vivo*, this work highlights the promising possibility of repurposing approved compounds for applications outside of their original use with the hope of streamlining the identification of new effective therapeutics against HIV.

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PP 1.11 – 00075

Selective nuclear retention of spliced and unspliced HIV-1 mRNAs following latency reversal

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Background: Following integration into the host genome and subsequent proviral transcription, HIV-1 uses alternative splicing to generate distinct viral RNA species belonging to three main classes: completely unspliced, singly spliced, and multiply spliced transcripts. The mechanisms permitting differential sequestration and nuclear export of unspliced and partially spliced HIV-1 mRNA transcripts (RRE-mRNA) is an understudied feature of HIV-1 persistence.

Methods: Using an in vitro primary T cell model of HIV-1 latency, we differentially tagged both the spliced and unspliced HIV-1 mRNA species with fluorescence probes. Proviral reactivation was monitored by a combination of Immuno-CasFISH and in situ RNA hybridization, and the nuclear-cytoplasmic distribution of transcripts was mapped. In parallel we quantified changes in the ratios of these species over time by direct long read RNA sequencing technology (Oxford Nanopore).

Results: Reactivation of cells latently infected with a single round HIV-1 reporter virus demonstrated that the perinucleolar zone is the initial site for Rev-RRE-mRNA assembly. Subsequently, the RNA-binding protein PTBP1 colocalized with both spliced and RRE-mRNA complexes in the perinucleolar zone. The Sam68 adaptor selectively associated with retained RRE-mRNA transcripts prior to export via the Rev/CRM1-dependent pathway. Direct RNA sequencing correlated with cellular RNA distributions and showed a predominance of multiply spliced messages including the Tat and Rev messages with fewer Env transcripts and even fewer full-length transcripts. Additionally, putative m6a modified bases were identified in the 5′ and 3′ UTR as well as in env and rev.

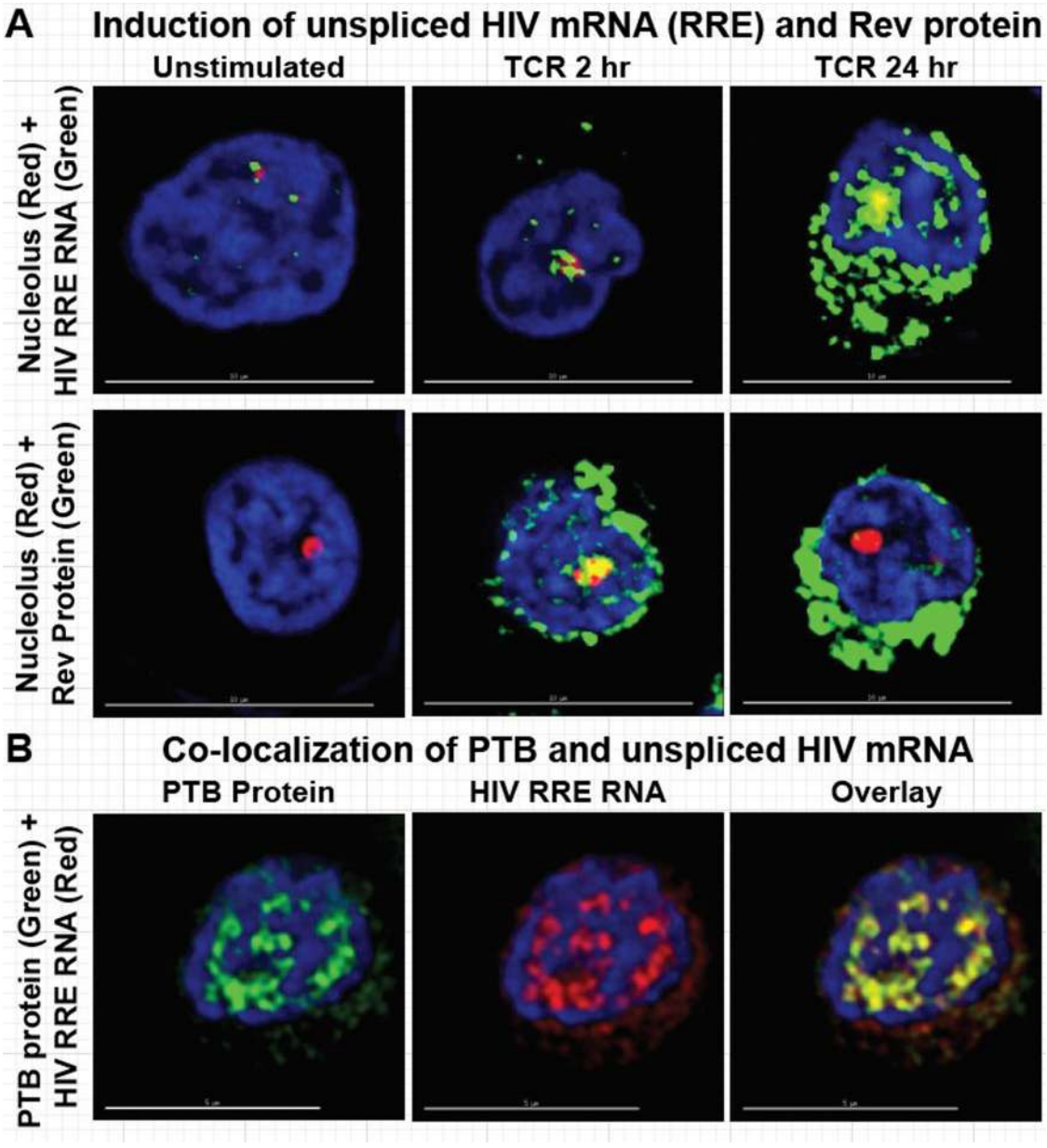

Figure 1 (abstract: PP 1.11–00075) Visualization of unspliced HIV mRNA and PTBP1

Conclusions: We showed that PTBP1 and Sam68 are the key adaptor proteins required for entry into the Rev/CRM1 nuclear export pathway. Our findings suggest that PTBP1 is exchanged with Sam68 prior to nuclear export. Using a combination of direct RNA sequencing technology and knockdown of PTBP1 and Sam68, we are analyzing the kinetics of HIV-1 RNA synthesis and stability following latency reversal, as well as identifying novel m6A RNA modification sites that distinguish differentially sorted transcripts. This work will be able to reveal the changes in viral transcription that occur post-reactivation and provide an explanation for the accumulation of spliced and unspliced mRNAs in latently infected cells.

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PP 1.12 – 00076

The lysine methyltransferase SMYD5 amplifies HIV-1 transcription and is post-transcriptionally upregulated by Tat and USP11

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Background: Despite great efforts, "shock and kill" approaches have so far failed to significantly reduce the size and impact of latent HIV-1 reservoirs. There is a need to develop alternative, possibly complementary, strategies to "shock and kill" to achieve durable viral control in the absence of antiretroviral therapy (ART). Transcriptional silencing in latency research is a relatively new concept. Many transcriptional regulators for HIV-1 have been identified. Besides histone deacetylation, a growing list of methyl transferases (MTs) and demethylases indicates that methylation of DNA, histones and non-histone proteins is essential for HIV-1 transcriptional regulation. Our working hypothesis is that co-activating MTs play a critical role in preventing permanent silencing of the HIV-1 locus. The rationale is that co-activating KMTs by methylating histones or Tat facilitate transcription initiating, thereby directly antagonizing repressive epigenetic mechanisms necessary for durable silencing of the HIV-1 locus.

Methods: We performed a comprehensive lentiviral shRNA screen of human lysine methyltransferases (KMTs) in J-Lat cells to identify new activators and repressors of HIV transcription. The top activating KMT SMYD5 was validated in primary CD4 + T cell experiments. To identify the mechanism underlying how SMYD5 contributes to HIV latency we performed luciferase assays, chromatin- and co-immunoprecipitation experiments, in vitro methylation and electrophoretic mobility shift (EMSA) assays.

Results: In an RNAi-based screen of human lysine methyltransferases we identified the SET and MYND domain-containing protein 5 (SMYD5), which was previously reported to target lysine 20 at histone H4 (H4K20me3), as a co-activator of HIV transcription. Knockdown of SMYD5 suppresses HIV-1 transcription in latently infected T-cell lines and primary CD4 + T cells. SMYD5 is recruited to the HIV-1 promoter upon activation, binds TAR RNA and interacts with Tat. We also provide evidence that SMYD5 is stabilized by Tat through the deubiquitinase USP11.

Conclusion: We report data that identify SMYD5 as new regulator of HIV transcription.

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PP 1.13 – 00078

CTL epitopes from structurally important hiv proteins are identified in rebound HIV

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Background: During an analytic treatment interruption (ATI), the elimination of HIV-infected cells by HIV-specific cytotoxic CD8 T-cells (CTLs) is required to control viral infection. However, unless treatment is initiated during acute infection, the HIV reservoir becomes dominated by CTL escape variants resistant to immune response. To address this issue, we applied a novel IMmunoinformatics Analysis Pipeline (IMAP) to identify CTL epitopes within structurally important HIV protein regions that avoid CTL immune escape mutations.

Methods: Employing the IMAP, we generated 8–14 mer overlapping peptides containing amino acid residues that are essential for HIV Gag protein structure/function. We excluded the peptides containing CTL escape mutations and those found within $<85\%$ of HIV-1 subtypes/recombinants. Using the Immune Epitope Database and NetMHCpan analysis tools, we selected the peptides that are predicted to be immunogenic and are binders to multiple HLA-I alleles. These peptides were compared to the Gag protein region extracted from HIV-RNA sequences of five participants who underwent multiple ATIs.

Results: We identified 32 overlapping peptides within the Gag protein from positions 150–166. Interestingly, when we compared this protein region to the Los Alamos HIV database, we observed 55% of the HIV sequences contained arginine (R) and 41% contained lysine (K) at position 154. Therefore, the R/K variant peptides combined ($n = 24$) were identified within 96% of viral sequences worldwide. of note, none of these peptides contained the R/K variations at HLA-I anchoring positions. Eight additional overlapping HIV peptides identified within positions 155–166 of the Gag protein region were found within 95% of HIV sequences globally. These 32 HIV peptides were predicted to bind to multiple HLA-I alleles, resulting in 88% worldwide population coverage. When including the R/K variant forms of the HIV peptides, 100% of plasma-derived HIV-RNA sequences from 5 participants, who experienced 3 separate treatment interruptions, contained our selected Gag peptides.

Conclusions: Applying the IMAP, we defined novel HIV peptides within a structurally important region of the Gag protein. These peptides were genetically conserved, avoided

known CTL escape mutations, and were identified within ATI-derived plasma HIV-RNA sequences, indicating these peptides as a pool are highly promising for eliciting a CD8 T-cell immune response during treatment interruption.

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PP 1.14 – 00094

Development of an immunocytochemistry assay to quantify the translationally active HIV reservoir

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Background: Although combination antiretroviral therapies (cART) efficiently suppress HIV replication, HIV persists in a long-lived reservoir and rebounds when cART is interrupted. The active reservoir consists of infected cells that transcribe HIV and produce viral proteins even in the presence of cART. These transcriptionally and translationally competent HIV-infected cells can contribute to chronic immune activation in people living with HIV on cART. Methods to quantify this active reservoir are needed to assess curative strategies.

Methods: An automated immunocytochemistry (ICC) assay coupled with computational image analysis was developed to detect and quantify intracellular Gag capsid protein (CA). Fixed cells were cytospinned onto microscopy slides and stained with antibodies against CA followed by image acquisition. We used nuclear staining to enumerate total cells and chromogenic signal to quantify the proportion of CA-positive cells. Digital ELISA and flow cytometry were used to validate CA-ICC with established assays.

Results: To determine the sensitivity of the CA-ICC assay we spiked the MoltIIIB cell line into uninfected Jurkat cells in limiting dilutions. Peripheral blood mononuclear cells (PMBCs) from HIV-seronegative donors before and after in vitro infection with an HIV laboratory strain were used to test the specificity of the staining. We applied this assay to detect HIV-1 p24- or SIV p27-containing cells in PBMCs from mouse and non-human primate animal models, respectively. We found that the proportion of CA positive cells from either animal models' PBMCs correlated with the pVL and cell-associated CA. We also applied this CA-ICC method to quantify the activity of small molecule targeted activators of cell kill in eliminating CA-expressing cells.

Conclusions: Here, we developed an ICC assay to study the active HIV-1 reservoir. Efforts are ongoing to further validate the method with PBMCs from HIV-seropositive donors on cART and provide a benchmark towards a functional HIV-1 cure.

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PP 1.15 – 00095

Proviruses persisting during the initial years of suppressive ART are relatively stable in terms of genetic diversity, clonal composition, and inferred integration date distribution

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Background: Elucidating on-ART proviral evolutionary dynamics can advance HIV cure efforts, but the genetic stability of the on-ART proviral pool remains incompletely understood. We reconstructed pre-ART within-host HIV evolutionary histories in six female seroconverters and leveraged this to characterize genetic diversity and inferred integration dates of proviruses sampled longitudinally on-ART.

Methods: A median 9 plasma samples/participant spanning a median of 7 years pre-ART, and a median 3 proviral samples/participant spanning a median 6 years during fully suppressive ART were studied. Plasma and proviral sequences were single-genome amplified *env-gp120*. To mitigate uncertainty in within-host phylogenetic reconstruction, a median 3185 (IQR:1445–4875) phylogenies were inferred per participant using Bayesian approaches. Proviral ages were estimated phylogenetically.

Results: Phylogenies were inferred from a median of 157 (IQR:67–193) pre-ART plasma *env* and 53 (IQR:45–99) on-ART proviral sequences/participant. In all participants, the distribution of proviral inferred integration dates persisting on ART

spanned nearly the entirety of untreated infection, and sequences dating to earlier infection were recovered in all participants. Nevertheless, the integration dates of on-ART proviruses were overall slightly skewed towards proviruses archived during chronic infection. The overall proportion of clonal proviruses ranged widely by participant (median 35%; range 6–45%) and increased in three of the five participants with longitudinal proviral sampling on-ART. Moreover, in all five participants, the same clone was recovered across multiple timepoints, including one instance 8 years apart, confirming long-term persistence of certain clones. In three participants, proviral integration date distribution remained stable over time. In one participant however the proviral population gradually shifted towards "younger" sequences that had integrated around the time of ART initiation. In another participant whose proviral sampling spanned 12 years, the on-ART proviral pool significantly shifted towards "older" sequences that had integrated earlier in infection.

Conclusions: Results extend estimates of on-ART reservoir decay rates inferred from longitudinal reservoir size measurements by providing insight into the underlying genetic composition and evolutionary dynamics of the proviruses persisting on-ART. Results confirm ongoing archiving of diverse within-host HIV lineages during untreated infection and their largely stable persistence, both in terms of overall genetic and clonal composition, during the initial years of ART.

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PP 1.16 – 00100

Single-cell multiomics analysis reveals distinct mechanisms of HIV persistence in memory CD4+ T cell subsets from tissues

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Background: We previously demonstrated using an ex vivo tonsil infection model that CCR5-tropic HIV (F4.HSA) fuses to and integrates into CD4+ T memory (Tm) cells expressing CD127 (alpha-chain of IL7 receptor). However, these cells poorly express the LTR-driven reporter gene HSA. By contrast, other Tm subsets efficiently support productive infection(1). Here, combining scRNAseq with CITE-seq for high-parameter surface phenotyping, we examine the transcriptomic and phenotypic features of productively- and non-productively-infected cells from HIV-exposed tonsils in this system.

Methods: Tonsil cells from 4 uninfected donors were mock-treated or exposed 4 days to F4.HSA, and then subjected to scRNAseq and 68-parameter CITE-seq. Productively-infected cells were identified as those expressing HSA protein (detectable by CITE-seq) and cells harboring HIV transcripts were identified from scRNAseq reads.

Results: We identified distinct populations of productively-infected (HSA+ and HIV RNA+) and transcriptionally-active but not productively-infected (HSA- and HIV RNA+) cells. Comparison of the transcriptomes and surface proteomes of these populations of infected cells revealed that HSA-RNA+ cells preferentially belong to the CD127+ Tm subset. This, together with observations that these cells harbor initiated, elongated, and completed transcripts but are blocked in HIV splicing(1), suggest a late block to HIV gene expression in infected CD127+ Tm cells. Relative to productively-infected cells, HSA-RNA+ cells express higher levels of antiviral factors IFIT3 and SOCS3 and preferentially belong to the central Tm subset. By contrast, productively-infected cells preferentially express activation markers (HLA-DR), Treg markers (CD25, CTLA4, Foxp3), and TNF receptor family members (GITR, Ox40). Interestingly, productively-infected cells also preferentially express MIR155RG, a microRNA previously shown to be upregulated on reactivated cells and which drives cells back into latency. This, together with our observation that other HIV transcriptional activators are downregulated [RN1] (SMYD5, UHRF1), suggests that productively-infected Tm cells from tonsils are being driven into a state of HIV latency.

Conclusions: HIV infection of tonsils establishes multiple populations of infected cells: transcriptionally-active reservoir cells which can be identified by CD127 expression, and productively-infected cells which are being driven to enter a state of HIV latency. These infected cells exhibit unique features which can reveal on mechanisms of persistence, and can serve as targets for viral eradication.

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PP 1.17 – 00103

The Ubiquitin Ligase ITCH and CPSF6 Control HIV Transcription

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Background: Pharmacological approaches to reactivate the latent HIV reservoir in vivo have been considered for more than a decade. However, the effectiveness of these approaches has been disappointing as only a minority of latently infected cells appear to be responsive to latency-reversing agents (LRA). A deeper understanding of the transcriptional regulation of the HIV-1 promoter is necessary before we can design highly effective LRAs.

Methods: To better understand the role CPSF6 (Cleavage and polyadenylation specificity factor 6) in viral transcription, we performed CRISPR/Cas9 mediated knock-out in primary T cells that were latently infected in vitro. We then evaluated the efficiency of viral reactivation in these cells.

Results: We found that CPSF6 controls the stability of protein phosphatase 2A (PP2A) subunit A, a phosphatase directly implicated in the de-phosphorylation and inactivation of CDK9 and RNA Pol II. Destruction of PP2A subunit A is effected by a complex of CPSF6 with the HECT-type E3 ligase known as ITCH. This complex recruits the PP2A scaffolding subunit A and de-destabilize it via the ubiquitin/proteasome system. Under normal, resting conditions, PP2A is therefore de-stabilized, allowing for high levels of HIV-1 transcriptional activity. CRISPR/Cas9-mediated knock-out of CPSF6 results in abnormal stabilization of PP2A, resulting in removal of the essential phosphate residues from CDK9 and RNA Pol II, which in turn severely hinders transcription.

Conclusions: The novel regulatory pathway that includes ITCH, CSF6 and PP2A provides a new avenue toward modulating HIV-1 transcription and highlights a potential gateway into latency. The availability of FDA-approved pharmacological agents to either activate or inhibit PP2A (currently in clinical

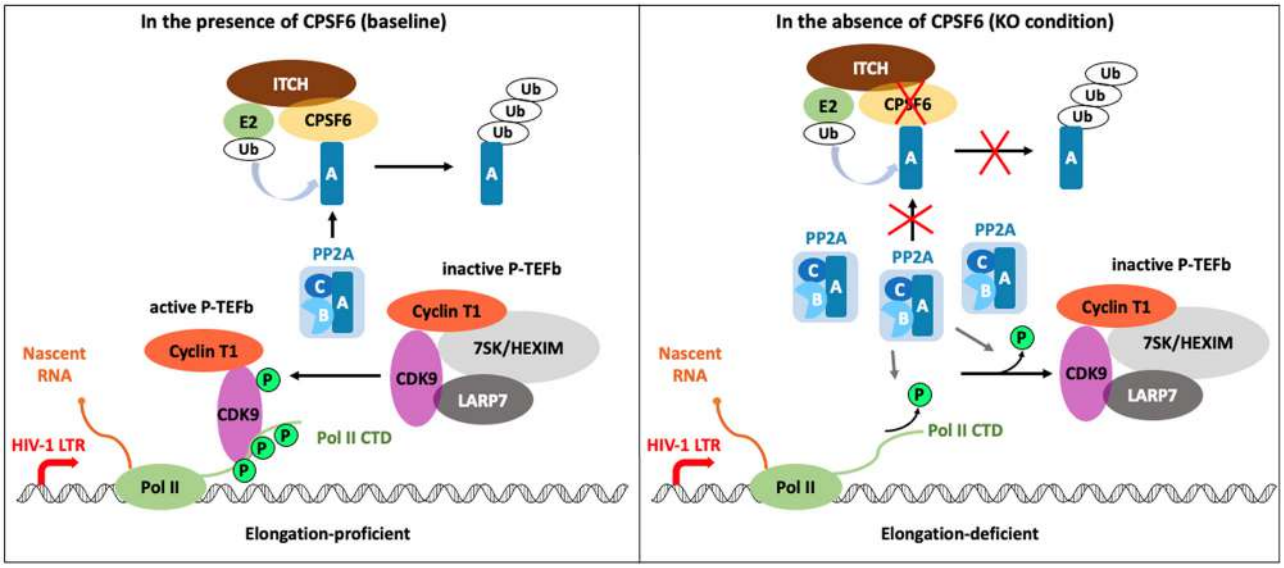

Figure 1 (abstract: PP 1.17–00103) ITCH, CPSF6 and PP2A control HIV transcription

trials against cancer) should lead to development of novel pharmacological strategies for manipulating viral transcription.

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PP 1.18 – 00105

Machine learning identifies differentiating physicochemical signatures between HIV subtypes in Nef domains associated with host-cell regulation

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Background: HIV subtype is associated with varied rates of disease progression. The HIV accessory protein, Nef, continues to be expressed during antiretroviral therapy (ART) where it has numerous intra- and extra-cellular immunoregulatory effects that may influence the development of pathologies associated with long-term ART. We analyzed Nef sequences from HIV subtypes A1, B, C, and D using a machine-learning approach that integrates functional amino acid information to identify unique physico-chemical features associated with Nef functional/structural domains for each HIV subtype.

Methods: 1628 non-identical sequences were obtained from the HIV database at Los Alamos representing subtypes A1, B, C, and D. Sequences were aligned and domains of interest identified. Following the generation of over 1000 features associated with amino acid physicochemical properties, we used statistical pruning and evolved neural networks to identify the top 5 Nef features associated with subtype differentiation. A signature pattern analysis was used to assess amino acids in domains that differentiated subtypes.

Results: Our feature-based domain scoring, followed by t-tests, identified subtype-specific domain-associated features. Subtype A was associated with alterations in Nef CD4 binding domain; subtype B was associated with alterations with the AP2 binding domain; subtype C was associated with alterations in the structural α-helix domain; and, subtype D was associated with alterations in the score $_{\beta}$-sheet A domain. Validation studies using evolved neural networks with domain-associated features as input differentiated subtype A (100%), subtype B (91%), subtype C (98.6%), and subtype D (89.4%). Specific domain-associated amino acids for each subtype were associated with substantial changes in hydrophobicity, polarity, and other structural-associated elements.

Conclusions: Previous studies have identified HIV Nef as one of the important drivers of immunoregulatory disease in HIV infected people on ART. Nef acts primarily on the trans-Golgi network and the internal cell membrane through interactions linked to the key features of the subtype-specific domains we identified with the ENN. The study supports the hypothesis that different Nef subtypes may contribute to subtype-specific disease progression.

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PP 1.19 – 00109

Differential decay dynamics of the inducible pool of HIV-1 infected CD4 + T cells and proviral DNA upon ART initiation revealed by the novel VIP-SPOT assay

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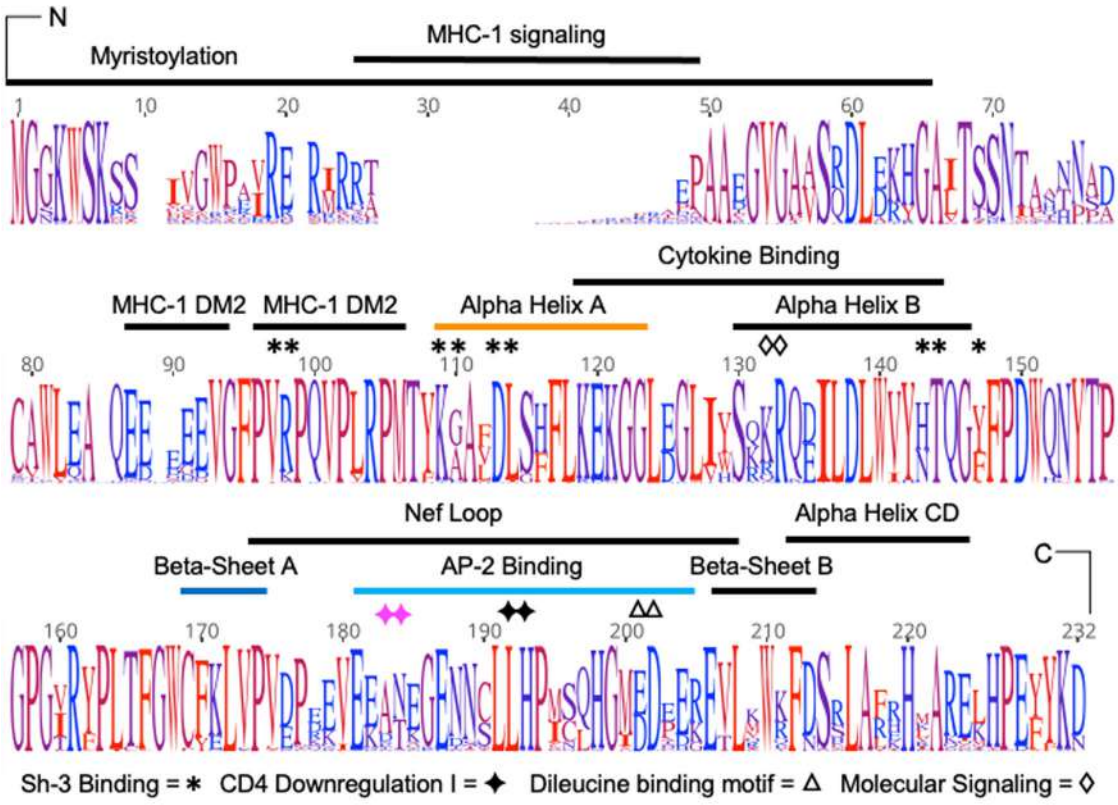
Background: Antiretroviral therapy (ART) supresses viral replication and this is reflected in a rapid decay of plasma viremia to levels below the limit of detection within a few weeks. Most productively infected cells die from cytopathic effects in the first few days, but a pool of latently-infected cells remains, establishing the viral reservoir, which fuels viral rebound whenever ART is discontinued. Understanding the establishment of this viral reservoir is key to designing effective HIV cure strategies.



SeqLogo consensus sequence showing Nef variation among subtypes and functional domains studied. The image represents the consensus alignment of 1628 sequences used in the study from HIV subtypes A1, B, C, and D. The height of each 1-letter amino acid code correlates with its presence in the aligned set of sequences. Amino acids are colored by hydrophobicity value, where red is the most hydrophobic and blue is the most hydrophilic. Our feature-based domain scoring and evolved neural network identified that Subtype A was associated with alterations in Nef CD4 binding domain (magenta); subtype B was associated with alterations with the AP2 binding domain (light blue); subtype C was associated with alterations in the structural α-helix domain (orange); and, subtype D was associated with alterations in the score β-sheet A domain (dark blue).

Figure 1 (abstract: PP 1.18–00105)

Methods: We compared the decay dynamics of the inducible pool of HIV-1 infected CD4+ T cells and total and intact HIV-1 DNA in 11 participants who started first-line ART with DTG +3TC or DTG+FTC/TAF in the setting of a clinical trial (EudraCT: 2019-002733-10). Using the VIP-SPOT assay, we measured the frequency of cells capable of reactivating in vitro and producing p24 viral protein at 0, 2, 4, 12, 24 and 48 weeks after ART initiation. DdPCR was used to quantify total HIV-1 DNA and intact proviral genomes in the same samples. Results analyses were performed blinded (i.e., without knowing within-study allocation).

Results: The proportion of the inducible pool of HIV-1 infected CD4+ T cells decayed rapidly after ART initiation, as evidenced by a median decrease of 92% in the first two weeks. Subsequently, the frequency of these HIV-1-producing cells remained fairly constant. In contrast, the frequency of circulating CD4+ T cells containing viral DNA or intact proviruses decreased with slower kinetics: HIV DNA decreased by 16% in the first 2 weeks of treatment, and at week 12 we observed a median decrease of 57% and 48% in total and intact proviruses, respectively.

Conclusions: The VIP-SPOT assay revealed that CD4+ T cells harboring inducible HIV-1 are rapidly and specifically cleared upon ART initiation, in contrast to the overall pool of cells containing either defective or intact proviral DNA. The specific mechanisms that target this fraction of infected CD4+ T cells are unknown. Future interventions that aim to impact the establishment of the viral reservoir could benefit from starting concomitantly with ART initiation to enhance their impact on the productive HIV-1 reservoir.

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PP 1.21 – 00118
Regulation of HIV-1 persistence by the CARD8 inflammasome

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Background: CARD8 is an innate immune sensor that can be activated through proteolytic cleavage of its N-terminal fragment. Recently, we reported that HIV-1 protease triggers CARD8 inflammasome activation and caspase-1-dependent pyroptosis. However, it remains unclear whether HIV-1 infection can activate the CARD8 inflammasome to trigger pyroptosis of

CD4$^+$ T cell and thereby modulates HIV-1 infection and persistence.

Methods: We co-cultured HIV-1-infected primary CD4$^+$ T cells with CFSE-labeled donor-matched PBMCs to assess infection of CD4$^+$ T cells and inflammasome activation. We also infected CD34-engrafted humanized mice to examine the role of CARD8 inflammasome in HIV persistence.

Results: We found that HIV-1 induces rapid CARD8-dependent pyroptosis of CD4$^+$ T cells in peripheral blood, tonsil tissues, and humanized mice. Mechanistically, the N-terminus of CARD8 is cleaved by HIV-1 protease encapsulated in the incoming viral particles immediately after viral entry, resulting in release of the bioactive C-terminal fragment for inflammasome assembly and pyroptosis of the host cells. The CARD8 inflammasome clears resting CD4$^+$ T cells post HIV entry and prevents reservoir establishment directly in these quiescent cells. By contrast, TCR signaling abolished CARD8 function through CARD8 degradation, allowing HIV-1 to complete reverse transcription and integration.

Conclusions: Our results demonstrate that the CARD8 inflammasome is activated post HIV-1 entry, which restricts both productive and latent infection in resting CD4$^+$ T cells. Our study provides critical insights into how CARD8 modulates HIV-1 infection and persistence.

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PP 1.22 – 00120

Suppression of CD4+ T-cell-intrinsic immunity by HIV-1 latency-reversing HDACi

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Background: "Shock-and-kill" is one of the conceptually most advanced strategies towards establishment of HIV-1 cure. Treatment with histone deacetylase inhibitors (HDACis) reactivates HIV-1 transcription *in vivo*, however, fails to significantly reduce the HIV-1 reservoir in infected individuals, indicating that it is insufficiently to eliminate latently infected cells. The impact of HDACis on the transcriptome and functionality of CD4$^+$ T-cells, the main HIV-1 reservoir *in vivo*, remains understudied.

Methods: Here, using scRNA-seq, we characterize HDACi treatment-induced alterations of CD4+ T-cell subpopulation-specific transcriptomes and correlation with HIV-1 reactivation from latency.

Results: Ex vivo exposure of CD4$^+$ T-cells from aviremic HIV-1-positive individuals with Panobinostat, but not Vorinostat, markedly reduced expression of genes and proteins required for T-cell-specific signalling and immunity, resulting in impaired TCR/CD28- and IL-2/PHA-triggered T-cell activation. Using latently infected J1.1 cells, we found that HDACi-treatment, but not the PKC modulator Bryostatin, efficiently blocks type I IFN-induced innate immune responses. Consequently, HIV-1 reactivation triggered by HDACi, in contrast to Bryostatin, was unsensitive to IFN-mediated reduction of reactivation. Finally, using sophisticated statistical analysis we identified genes significantly differentially expressed in HIV-1-RNA-positive CD4$^+$ T-cells compared to the HIV-1-RNA-negative cells from the same donor, independent of the LRA used.

Conclusions: In summary, we show that HDACi treatment dampens CD4$^+$ T-cell functionality, that may contribute to the limited reduction of the latent reservoir after HDACi-treatment *in vivo*. Further, we identified an HIV-1 reactivation-specific gene signature that correlates with HIV-1 RNA abundance in reactivating CD4$^+$ T-cells from aviremic donors that may also serve as a surrogate for reactivating cells *in vivo*.

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PP 1.23 – 00130

Long-read sequencing assay allows accurate characterization of the HIV-1 reservoir

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Background: Current near full-length (NFL) HIV-1 proviral genome sequencing assays are based on labor-intensive and costly principles of repeated PCRs at limiting dilution, restricting their scalability. We developed a long-read sequencing assay that allows for high-throughput amplicon sequencing of NFL HIV-1 genomes.

Methods: The assay was performed on peripheral blood CD4 T cells from 18 chronic ART-suppressed individuals. HIV-1 reservoir sizes were assessed by ddPCR and ranged from 322 to 4869 total HIV-1 DNA copies/million CD4 T cells. For each participant, 6 PCR replicates with each 500 ng DNA input were run. By tagging individual HIV-1 genomes with two distinct unique molecular identifiers (UMIs), the step of limiting dilution can be omitted, enabling long-range PCR amplification of many NFL genomes in a single reaction. Following long-read sequencing on an Oxford Nanopore MinION, UMI-based demultiplexing allowed for the construction of highly accurate consensus genomes, while excluding chimeric PCR artefacts.

Results: The long-read assay yielded an average of 15 distinct HIV-1 proviruses per PCR replicate, per participant (range: 3–55). In total, 1308 distinct proviruses were retrieved, with 5% being putatively intact which corresponds to previously reported numbers. Additional benchmarking revealed (1) a detection efficiency of 13 ± 9% by dividing the number of distinct proviruses by the total number of input HIV-1 copies and (2) a consensus accuracy of 99.99% when compared to reference

genomes acquired by traditional NFL assays, with only a few errors remaining in homopolymeric regions.

Conclusions: The long-read assay enables high-throughput, scalable characterization of the proviral landscape which could have wide future applicability in the field of HIV-1 research to further increase our understanding on HIV-1 reservoir composition and dynamics.

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PP 1.24 – 00132

BET PROTACS Reveal BRD4 Disruption of the 7SK/P-TEFb Equilibrium is Critical for Effective Reactivation of Latent HIV in CD4+ T-cells

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Background: PROTACs (proteolysis-targeting chimeras) are bifunctional molecules that simultaneously bind an E3 ubiquitin ligase and a protein of interest (POI), driving ubiquitination of the POI by the E3 ligase complex and subsequent degradation by the proteasome. Pan-inhibitors of the BET (bromo and extra-terminal domain) family of proteins (BETi) are known HIV latency reversal agents, however of the 4 family members, only BRD4 is thought to have a significant role in maintenance of latency. Here we examine the ability of various BET-PROTACs to specifically degrade BRD4 and induce latency reversal.

Methods: BET PROTACs were assess for BRD4-specific degradation by western blots. Latency reversal and synergy with IAPi inhibitors in Jurkat models and in primary CD4+ T-cells was assessed by flow cytometry and cell-associated RNA assays respectively. HEXIM1 protein induction was measured by western blot and transcription induction via a HEXIM1 promoter driven luciferase reporter assay.

Results: BET-targeted PROTACs MZ1 and ZXH 3-26 induce BRD4-specific degradation in both Jurkat latency models and primary CD4+ T-cells. Despite targeted degradation, both PROTACs failed to induce cell-associated viral RNA in cells from durably suppressed individuals as compared to pan-BETi JQ1. Further, PROTAC-mediated degradation in combination with IAP inhibitor AZD5582, which we have previously shown synergizes with BETi for latency reversal, failed to further increase HIV transcription. These results reveal a critical function for BRD4 in latency reversal that is independent of BD domain antagonism. We studied traditional BRD4 interacting partners and generated various mutant forms of BRD4 for overexpression in latency models. We observed BRD4 degradation fails to upregulate HEXIM1 transcription and protein levels, a key difference compared to BET inhibitors.

Conclusions: HEXIM is a critical component of the 7SK snRNA complex which is responsible for regulation of elongation factor P-TEFb. HEXIM upregulation is a well characterized response to BET antagonists and other small molecule inhibitors which disrupt the 7SK/P-TEFb equilibrium. These observations suggest BETi mediated disruption of P-TEFb is dependent on the BRD4 protein, independent of the BD domains, and in primary CD4+ T-cells is a key factor in latency reversal of HIV by BETi.

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Daily Variations in Residual Viral Transcription in ART-Treated People Living with HIV-1

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Acknowledgment: Amelie Pagliuzza (CR-CHUM), Rémi Fromentin (CR-CHUM) and Nicolas Chomont (UdeM, CR-CHUM) for their contributions in the PCR/RT-PCR to measure HIV-1 transcription levels.

Background: Biological functions fluctuate in a circadian manner to align with environmental changes. Daily variations are observed for plasma cortisol and melatonin, with a lower CD4 count observed in the morning. HIV-1 infection is characterized by profound alterations in CD4+ T-cell homeostasis, combined with chronic immune activation and gut barrier damage. Daily variations in immunological/virological parameters in people living with HIV-1 (PLWH) receiving antiretroviral therapy (ART) remain poorly investigated.

Methods: Eleven ART-treated PLWH (age: 57 years old; median CD4 counts: 606 cells/μl; time since infection: 242 months; aviremia under ART: 216 months) were admitted for 40 hours and blood was collected, before food intake, every 4 hours, for 24 hours (8am, 12am, 4pm, 8pm, 12pm, 4am, 8am). Plasma levels of cortisol/melatonin and markers of mucosal barrier permeability (FABP2, LBP) were measured by ELISA. Flow cytometry allowed the quantification and phenotypic characterization of blood leukocyte subsets. Total CD4+ T-cells were isolated and matched RNA/DNA isolated by dual extraction served to quantify cell-associated integrated HIV-DNA and LTR-Gag HIV-RNA levels by nested PCR/RT-PCR. Peak/nadir term was used to define maximal/minimal levels.

Results: Plasma cortisol and melatonin levels peaked at 8am and 4am, respectively. Peak plasma FABP2 levels at 4am coincided with nadir LBP levels at 4am. For memory/naive/regulatory CD4+ T-cells, peak counts were observed between 8pm-4am, while nadir counts at 12pm. The expression of the HIV-1 co-receptors CCR5/CXCR4, gut-homing molecules CCR6/integrin b7, and the immune checkpoint inhibitor PD-1 on memory T-cells expression levels peaked between 8pm-4am. Nadir non-classical monocyte counts were observed at 4am. Integrated HIV-DNA levels in CD4+ T-cells demonstrated donor-to-donor variations but minor fluctuations over the 24 hours of sampling. In contrast, the HIV-RNA/DNA ratios (surrogate marker of HIV-1 transcription) peaked at 4am.

Conclusions: Daily variations in melatonin/cortisol levels, T-cell/myeloid counts, mucosal permeability markers, key

molecules involved in HIV replication/pathogenesis, as well as in circulating replication-competent viral reservoir, were observed in ART-treated PLWH. The peak of HIV transcription in CD4 + T–cells, observed at 4am, coincided with the peak FABP2 and melatonin and nadir LBP levels. These findings should inform therapeutic interventions in HIV-1 cure/remission on the importance of selecting the optimal time of treatment/monitoring.

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PP 1.26 – 00143

Understanding HIV transcription in Kaposi's sarcoma tumors during antiretroviral therapy

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Background: Epidemic Kaposi's sarcoma (KS), characterized by co-infection with HHV-8 and HIV, is a major cause of mortality in sub-Saharan Africa. Antiretroviral therapy (ART) significantly reduces the risk of developing KS, and for those with KS, tumors frequently resolve with ART alone. However, for unknown reasons, a significant number of KS cases do not resolve and can progress to death despite improved CD4 + T-cell counts and undetectable plasma HIV viral loads. To explore how HIV transcription responds to ART in the KS tumor microenvironment, we designed a novel approach to sequence HIV spliced transcripts found in the blood and tumors of study participants on ART with persistent KS.

Methods: We sequenced HIV found in DNA and RNA isolated from plasma, PBMCs, and tumor biopsies, before and after ART, in four Ugandan study participants who had unresponsive or progressive KS after 180–250 days of ART. We used two different limiting dilution approaches: One with *env-nef* primers and the other using primers targeting the 5′ and 3′ untranslated regions, specifically designed to amplify near full-length HIV spliced transcripts from isolated RNA. We inferred maximum-likelihood phylogenies by focusing on the Exon 7 region of the HIV genome, which all the sequences shared.

Results: Using our new sequencing approach, we found HIV-spliced transcripts in RNA from both plasma and tumor samples and determined their protein-coding potential by characterizing their splicing patterns. While HIV transcripts were not amplified from plasma and PBMCs after ART, HIV RNA expression in KS tumors is maintained, but frequently featured hypervariable sequence or defective splicing usually resulting in *gag-nef* fusions.

Conclusions: Overall, our results demonstrated that HIV located in KS tumors continues to be transcriptionally active, even after ART has reduced plasma HIV viral load to undetectable levels and restored immune function, which could influence tumor maintenance and progression. Continued studies using a larger cohort and additional methods that define the HIV transcriptionally active reservoir in the KS tumor niche could lead to novel therapies for KS.

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PP 1.27 – 00147

Role of the pol gene enhancer in HIV-1 transcription and replication in myeloid infected cells

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Background: There is increasing evidence of the physiological relevance of myeloid HIV-1 reservoirs such as brain microglia and urethral macrophages. However, the molecular mechanisms of HIV-1 gene expression in myeloid infected cells are still poorly understood. The HIV-1 intragenic cis-regulatory region (IRR) located in the pol gene exhibits an enhancer activity on the 5′LTR promoter. The IRR possesses multiple binding sites for various cellular transcription factors. Here, we characterized several of these binding sites and their functional involvement in the IRR-mediated control of HIV-1 gene expression in monocytes/macrophages. Special emphasis was put on studying several binding sites for the myeloid PU.1 transcription factor, known to be a pioneer factor inducing the opening of heterochromatin in enhancers.

Methods: ChIP-qPCR, p24 ELISA, purification of primary cells from blood samples and infection studies.

Results: We demonstrated the in vivo recruitment of PU.1 to the HIV-1 intragenic enhancer in latently-infected cell lines from myeloid origin. We physically characterized in vitro PU.1 binding to the different intragenic PU-boxes and identified mutations abolishing PU.1 binding without altering the underlying amino acid sequence of the pol gene. We demonstrated the role of the PU-boxes in the enhancer activity of the IRR and in its epigenetic profile in latency and reactivated conditions, in concert with other IRR transcription factor binding sites. We showed the importance of intragenic transcription factor binding sites in HIV-1 replication using mutated viral particles in single-round infection experiments using primary monocytes-derived macrophages isolated from uninfected individuals. To overcome HIV- 1 persistence, targeted approaches for each specific reservoir are needed. As a proof-of-concept, we revealed the potential therapeutic application of a specific inhibitor interfering with PU.1 binding as a new anti-HIV-1 approach.

Conclusions: The HIV-1 intragenic enhancer brings an additional factor in an already complex network of regulators affecting the level of HIV-1 transcription. Such complexity could allow a finer-tuned regulation that might find its purpose when HIV-1 transcription needs to be moderately or transiently modified within different cellular and chromatin environments. The role of the IRR in HIV-1 gene expression regulation opens new avenues for HIV cure approaches targeting the heterogeneous cellular and tissue latent reservoirs of virus.

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PP 1.28 – 00156

The HIV-1 antisense RNA Ast promotes viral latency via epigenetic silencing of the proviral 5′LTR and is expressed in latently infected cells from ART-suppressed donors

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Background: HIV-1 expresses a 2.6-kb antisense transcript (*Ast*) with protein-coding and noncoding roles. We previously showed that *Ast* promotes HIV-1 latency by recruiting the PRC2 complex to the 5′LTR leading to deposition of H3K27me3 marks and epigenetic silencing. We sought to identify *Ast* domains and motifs involved in these effects. We also assessed *Ast* expression in resting CD4+ T cells from ART-suppressed PLWH.

Methods: Functional activity of *Ast* mutants was assessed via stable transduction in the Jurkat E4 latency model. ChIP, ChIRP and RIP assays were used to assess the interaction between *Ast* and its binding partners. Expression of *Ast* in patient-derived samples was assessed by CARD-SGS and by digital PCR.

Results: The 5′ end of *Ast* mapping in the U3 region of the 3′ LTR (U3AST) interacts with the homologous U3 region of the proviral 5′LTR. Nucleotide substitutions in two pyrimidine-rich motifs within U3AST disrupted interaction between *Ast* and 5′ LTR. Over-expression of the U3AST fragment in latently infected cells reversed latency by displacing endogenous *Ast* from the 5′ LTR. A G-quadruplex (G4) motif at the center of *Ast* mediates interaction with PRC2. Mutation of 70 nt comprising the G4 motif abolished the interaction with PRC2 and the latency-promoting function of *Ast*. Full suppressive activity requires interaction with additional host factors, including the transcriptional silencers YY1 and CTCF, and members of the chromatin remodeling complexes BAF and NuRD. Substitution of a domain at the 3′ of *Ast* abolished the interaction with these host factors and latency-promoting function of *Ast*. CARD-SGS analyses detected on average 1 copy of *Ast* in ~5% of infected cells from donors on ART, whereas more sensitive digital PCR detected *Ast* in ~26% of infected cells.

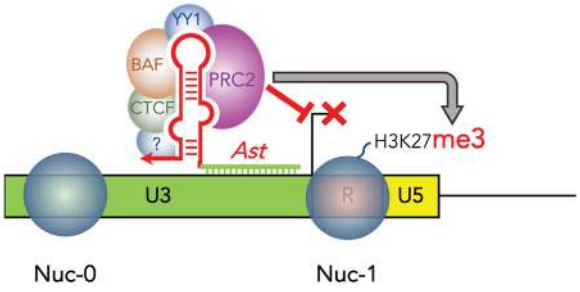

Figure. Ast model

Conclusion: The HIV-1 *Ast* promotes viral latency in vitro via direct recruitment of transcriptional silencers and chromatin remodeling complexes to HIV-1 5′LTR, supporting its use in cure strategies. Expression of *Ast* is detectable in latently infected CD4+ T cells from ART-suppressed PLWH.

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PP 1.29 – 00158

The chaperone protein p32 stabilizes HIV-1 Tat and strengthens the p-TEFb/RNAPII/TAR complex promoting HIV transcription elongation

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Background: HIV gene expression is modulated by the combinatorial activity of the HIV transcriptional activator, Tat, host transcription factors and chromatin remodeling complexes.

Methods: To identify novel host factors regulating HIV transcription we used specific single guide RNAs (sgRNAs) and endonuclease deficient Cas9 (dCAS9) to perform chromatin affinity purification of the integrated HIV promoter followed by mass spectrometry (ChAP-MS).

Results: The scaffold protein, p32, also called ASF/SF2 splicing factor-associated protein, was identified among the top enriched factors present in actively transcribing HIV promoters but absent in silenced ones. Chromatin immunoprecipitation analysis confirmed the presence of p32 on active HIV promoters and its enhanced recruitment by Tat. HIV uses Tat to efficiently recruit P-TEFb (CDK9/CCNT1) to TAR, an RNA secondary structure that forms at the first 59 bp of HIV transcripts, to enhance RNAPII transcriptional elongation. The RNA interference of p32 significantly reduced HIV transcription in primary CD4+ T cells and in HIV chronically infected cells, independently of either HIV splicing or p32 anti-splicing activity. Conversely, overexpression of p32 specifically increased Tat-dependent HIV transcription. p32 was found to directly interact with Tat's basic domain enhancing Tat stability and half-life. Conversely, p32 associates with Tat via N- and C- terminal domains. Likely due its scaffold properties, p32 also promoted Tat association with TAR, p-TEFb and RNAPII enhancing Tat-dependent HIV transcription.

Conclusions: In sum, we identified p32 as a novel host factor that interacts and stabilizes Tat protein, promotes Tat-dependent transcriptional regulation, and may be explored for HIV targeted transcriptional inhibition.

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PP 1.30 – 00159

Dating HIV-1 reservoir formation in ARV-suppressed Ugandans

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Background: A small but stable population of latently infected cells, predominantly CD4 + T cells, allows long-term persistence of replication-competent proviruses despite antiretroviral therapy, precluding a cure. The timing of the latent viral reservoir (LVR) establishment is of interest, due to evidence that suggest proviruses are preferentially archived at the time of ART initiation. We used bayroot, a Bayesian extension of root-to-tip regression, to determine temporal patterns of LVR establishment in a Ugandan cohort.

Methods: The reverse transcriptase region of pol and the gp41 region of env were deep sequenced from Quantitative viral outgrowth assays (QVOA) and pre-ART plasma of Ugandans living with HIV-1, who were virally suppressed on ART for > 1 year with known seroconversion windows and at least two archived ART naïve plasma samples. Maximum likelihood phylogenies were reconstructed and rooted using root-to-tip regression, with sampling dates of reservoir sequences censored. The rooted tree was the input for bayroot, constraining the root date to the seroconversion window, and integration date estimates to between seroconversion and ART initiation. This method was also applied to estimate integration dates using previously published data from HIV-infected South African women (CAPRISA cohort).

Results: Participants in the Ugandan cohort (n = 11) were observed for a median of 6.5 (IQR 5.7–8.3) and 10.6 (IQR 7.5–11.7) years before and after ART initiation, respectively. Most of the Ugandans (10/11) were infected with HIV-1 subtype D, and 87.9% of the pre-ART and 56.3% of the viral outgrowth sequences were unique (CI = 77.4–98.4 and 39.8–72.8% respectively). Integration dates were estimated to be relatively evenly distributed throughout viremia in 9/11 Ugandan participants. In contrast, in the CAPRISA cohort, sequences were more commonly estimated to have entered the LVR close to ART initiation, as previously reported.

Conclusions: Timing of LVR establishment is variable between populations. Interventions to limit LVR establishment around the time of ART initiation may have variable individual-level effects.

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PP 1.31 – 00161

Chimeric proviral/human transcription events at the BACH2 integration locus in cellular models for chronic HIV infection

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Background: Chronic HIV-1 infection is characterized by accumulation of proviral sequences in the genomes of HIV target cells. Integration of viral-derived DNA is found at preferential loci, suggesting site-specific crosstalk between viral sequences and human genes. This crosstalk has been postulated to play a role in emergence of clonal infected cell populations. One prominent locus for viral/human crosstalk is the BTB Domain and CNC Homolog 2 (BACH2) gene. Up to 40% of people living with HIV (PLWH) are thought to carry proviral sequence integrations in BACH2. Studies indicate that these integrations alter cell physiology through LTR exaptation and aberrant splicing events.

Methods: To decipher HIV/BACH2 crosstalk, we have used CRISPR/Cas9-based genome engineering to generate several cell models with BACH2-HIV-1 reporter integrants. Clonal lines were verified extensively for correct targeting and analysed for LTR activity, inducibility and epigenetic regulation. Furthermore, we used targeted transcriptional activation to decipher LTR/BACH2 crosstalk at transcriptomic level.

Results: Using cell line models that mimic proviral BACH2 integrations observed in PLWH, we show that LTR transcriptional activity is repressed in BACH2 intronic regions associated with integrations in vivo. This repression is not observed in regions devoid of in vivo integrations. We demonstrate that these findings are reflected in epigenetic modifications on the LTR. Furthermore, we studied the emergence of LTR/BACH2 chimeric transcription events, that have previously been reported to occur in vivo. We demonstrate how chimeric transcription is influenced by latency reversing agents or compounds proclaimed to supress LTR-derived gene expression. Finally, we used transcriptome analysis to define targets of LTR/BACH2 chimeric transcripts.

Conclusions: Our study provides first mechanistic insight into features of proviral/BACH2 crosstalk that might contribute to our understanding of pathogenic effects observed in PLWH on

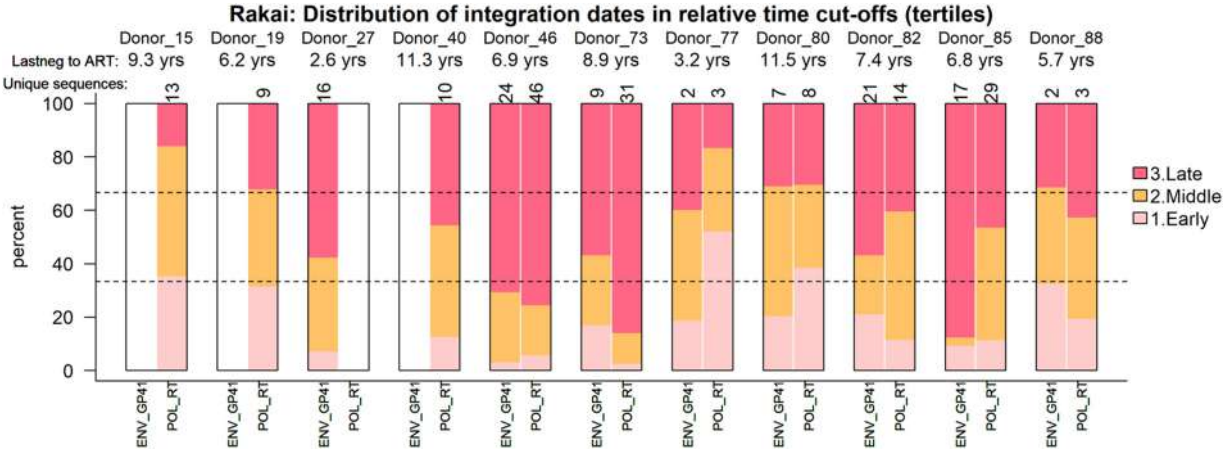

Figure 1 (abstract: PP 1.30–00159) Distribution of integration dates in tertiles

ART, such as clonal expansion of infected cell and chronic immune activation.

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PP 1.32 – 00163

cGAS/STING Signaling Drives HIV-1 Replication in Acutely Infected Macrophages

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Background: Macrophages play an important role in HIV-1 pathogenesis *in vivo*, including serving as a major viral reservoir that contributes to viral persistence during antiretroviral therapy. The determinants that govern HIV-1 replication in macrophages are not well understood. We hypothesize that recognition of double stranded DNA by cGAS/STING drives NF-kB-dependent HIV-1 replication at early time points after viral infection of macrophages.

Methods: Monocyte-derived macrophages (MDMs) infected with HIV-1 were used to evaluate the effects of NF-kB and cGAS/STING inhibition on viral replication and transcription. Western blot and ELISA analyses were used to evaluate NF-kB translocation to the nucleus in response to viral infection. Chromatin immunoprecipitation (ChIP) analysis was used to evaluate transcription factor recruitment to the viral promoter. Single cell RNA sequencing (scRNA-Seq) was used to evaluate transcriptomic changes in macrophages in response to HIV-1 infection.

Results: We demonstrated that shortly following infection with HIV-1, the p65 subunit of NF-kB translocates to the nucleus and associates with the HIV-1 promoter. The inhibition of NF-kB signaling, but not NFAT or AP-1 signaling, was sufficient to prevent HIV-1 replication in MDMs. The vital role of NF-kB signaling in HIV-1 replication in MDMs was confirmed by scRNA-Seq that demonstrated a significant increase in NF-kB activity in infected cells. Inhibition of cGAS/STING signaling led to a significant decrease in HIV-1 replication in MDMs, whereas inhibition of other innate immune receptors did not. Conversely, activation of cGAS/STING signaling in infected MDMs led to an increase in viral replication in an interferon and NF-kB-dependent manner. Finally, knockdown of STING using shRNA led to a significant decrease in HIV-1 replication in acutely infected MDMs. Together, our data suggest that cGAS/STING-mediated activation of NF-kB is necessary for viral replication in acutely infected macrophages.

Conclusions: Our studies implicate the cGAS/STING/NF-kB signaling axis as a driver of HIV-1 replication in acutely infected macrophages and suggest that this pathway might serve as a target to prevent the establishment of the macrophage reservoir.

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PP 1.33 – 00167

Integrated single-cell multi-omic profiling of HIV latency reversal

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Background: Despite the substantial success of antiretroviral therapy, HIV cannot be cured because of the persistence of stable cellular latent reservoirs, a major obstacle for the complete eradication of HIV. A deeper understanding of the mechanisms of transcriptional and epigenetic regulation of HIV latency will be required to fully reactivate HIV gene expression and ultimately deplete the reservoirs.

Methods: To understand the molecular mechanism of latently infected cells, we employed an integrated single-cell RNA-seq and ATAC-seq approach to simultaneously profile the transcriptomic and epigenomic landscape of latently infected cells partially reactivated with three latency-reversing agents (LRAs) with different mechanisms of action, Prostratin (PKC activator), iBET151 (Bromodomain inhibitor) and Vorinostat (HDAC inhibitor). Differentially expressed genes from scRNA-seq and differentially accessible chromatin peaks from scATAC-seq were examined for perturbation of known transcriptional pathways and enrichment of specific transcription factor (TF) binding sites in differentially open chromatin regions, respectively.

Results: We obtained high-quality multi-omic profiles of ~40 000 HIV-infected primary CD4 cells. Across the dataset we observed that reactivation of HIV transcription was highly correlated with proviral accessibility. From these data we were able to define set of host cell transcripts and TFs, whose expression or activity was correlated with viral gene expression. Furthermore, we demonstrate that a multivariate machine-learning model trained on these data was 72% accurate at predicting viral reactivation. In addition, we validated the role of a new candidate HIV-regulating factor, GATA3, in the viral response to prostratin.

Conclusions: In summary, we have demonstrated the power of integrated multi-modal single-cell analysis to uncover novel relationships between host cell transcripts, chromatin accessibility, and TF dynamics in HIV infected cells. These methods will likely lead to further insights and the identification of novel targets for latency-reversing agents.

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PP 1.34 – 00170

Intestinal endothelial cells substantially increase HIV infection and latency in resting and activated CD4+ T cells, particularly affecting CCR6+ Th17 subpopulation

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Background: With suppressive antiretroviral therapy, HIV infection is well managed in most patients. However, eradication and cure are still beyond reach, and lifelong therapy is required. The primary reason is due to latent viral reservoirs especially in CD4+ T cells. In untreated patients as well as most successfully treated patients there are intestinal pathology including massive depletion of T helper cells and bacterial translocation. Th17 cells in the intestinal mucosal area are particularly affected. Latent reservoir in CD4+ T cells are also found in gut associated lymphatic tissues. In this study we examined intestinal endothelial cells for their impact on HIV infection and latency in CD4+ T cells.

Methods: CD4+ T cells from peripheral blood were isolated and co-cultured with intestinal endothelial cells. A GFP expressing reporter virus was used to infect T cells cultured alone or cultured with endothelial cells. Productive infection was examined 6 days post infection, while latent infection was examined by sorting GFP negative cells on day 8 post infection and stimulating with and without PMA/Ionomycin.

Results: We found that intestinal endothelial cells dramatically increased HIV infection in resting CD4+ T helper cells, in productive infection as well as latent infection. In activated CD4+ T cells, endothelial cells enabled the formation of latent infection in addition to the increase of productive infection. Endothelial-cell-mediated HIV infection involved integrins (ICAM and VCAM) and the cytokine IL-6. CCR6+ Th17 subpopulation was particularly susceptible to such endothelial cell promoted infection.

Conclusions: Endothelial cells, which are widely present in lymphoid tissues including intestinal mucosal area and interact regularly with T cells physiologically, significantly increase HIV infection and latent reservoir formation in CD4+ T cells, particularly in CCR6+ Th17 cells. Our study highlighted the importance of endothelial cells and the tissue environment in HIV pathology and persistence.

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PP 1.35 – 00172

A histone deacetylase network regulates epigenetic reprogramming and viral silencing in HIV infected cells

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Background: During HIV infection, a latent viral reservoir is formed in CD4 T cells that persists during antiretroviral therapy (ART) and is maintained by a heritable state of transcriptional repression. Recent findings indicate that approximately 70% of the latent reservoir originates from infections occurring in the months near the time of ART initiation, raising the possibility that interventions during this period might prevent reservoir seeding and reduce reservoir size. Based on this concept, we tested the ability of compounds that target epigenetic machinery to prevent the establishment of latent HIV infection in primary CD4 T cells.

Methods: HIV latency is modeled in primary CD4+ T cells isolated from healthy donors. T cells are activated by TCR stimulation and infected with HIV-NL4-3Δ6-dreGFP/Thy1.2 virus. Infected T cells were cultured with standard tissue culture practices in the presence of IL7 and IL2 and monitored by flow cytometry for viral gene expression (GFP/Thy1.2) and for immune surface markers to identify immune cell subsets. Protein expression was assessed by immunoblot analysis and histone mark binding to genomic/viral targets was accomplished using CUT&RUN. Genetic manipulations were accomplished by electroporating with CRISPR-CAS9 ribonucleoproteins directed against targets of interest.

Results: We identified class 1 histone deacetylase inhibitors (HDACi) as potent agents of latency prevention, an activity distinct from latency reversal. Inhibiting HDACs in productively infected cells caused extended maintenance of HIV expression, even after HDACi withdrawal, and this activity was associated with persistently elevated H3K9 acetylation and reduced H3K9 methylation at the viral LTR promoter region. HDAC inhibition in HIV-infected CD4 T cells during effector-to-memory transition also led to changes in the memory phenotype of infected cells. Through knockout of individual HDACs and use of HDAC-selective inhibitors, we determined that HDAC1/2 and HDAC3 play crucial and distinct roles in proviral silencing initiation.

Conclusions: Overall, this work indicates that a network of HDACs regulate a critical gateway process for HIV latency establishment and are required for the development of CD4 T-cell memory subsets that preferentially harbor long-lived, latent provirus. Epigenetic reprogramming by clinical targeting of HDACs during ART initiation may represent a novel way to prevent seeding of the HIV reservoir in vivo.

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PP 1.36 – 00182

Effect of HIV-1C Transmitted/Founder Viruses 5′ LTR and tat Genetic Variation on Viral Reservoir Size and Latency Reversal Potential

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Background: Persistence of the latent viral reservoir presents a major block to HIV-1 cure. However, the mechanisms that govern HIV-1 latency are unknown. The viral promotor, long terminal repeat (LTR), drives viral transcription and is enhanced by Transactivator of transcription (Tat). Studies have reported inter- and intra- subtype LTR and tat genetic variation which translates into functional differences. However, the effect of this genetic variation on viral latency development or reversal is unknown, hence this study seeks to address this. Furthermore, the only HIV

latency model available is subtype B based, referred to as JLAT. On the other hand, HIV-1C is responsible for approximately 46% of global HIV infections and is predominant in sub-Saharan Africa. Therefore, this study undertook to establish a HIV-1C latency model, C-JLAT.

Methods: A minimal genome reporter virus (termed "C731CC") was constructed using HIV-1C consensus LTR and Tat. Jurkat cells were infected with C731CC and subsequently GFP- cells representing true negative and latent cells were sorted by FACS. These GFP- cells were stimulated with phorbol 12-myristate 13-acetate (PMA) and cells expressing GFP after stimulation were then FACS sorted and cultured until they reverted back to a latent stage. HIV-1B and HIV-1C Tat expression was measured by western blot. The reactivation potential of HIV-1 subtype B and C was determined by reactivating JLAT vs. C-JLAT.

Results: Our data demonstrate that JLAT and C-JLAT express the same levels of Tat. Interestingly, HIV-1B was twice as more sensitive to stimulation with PMA, compared to subtype C.

Conclusions: Taken together our data suggests that subtype C has a higher propensity for establishing a more latent provirus than subtype B. Ongoing studies involve measuring the amount of integrated copies with Alu-gag PCR in both subtypes, as well as replacing the consensus HIV-1C LTR and Tat in C731CC with patient-derived sequences. These viruses will subsequently be used to infect Jurkat cells to measure the reactivation potential of the different patient viruses with different latency reversing agents.

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PP 1.37 – 00186

Investigating Short-Term Effects of COVID-19 mRNA Vaccination on Plasma Viremia and Intact HIV Reservoir Size in Individuals Receiving Antiretroviral Therapy (ART)

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Background: Anecdotal reports of transient HIV viremia in ART-suppressed individuals following COVID-19 mRNA vaccination suggest a possible stimulatory effect on the HIV reservoir. This was further supported by a recent study demonstrating that Nef-specific CD8+ T cells increased and acquired granzyme-B effector function following BNT162b2 vaccination, and that this correlated with markers of immune-mediated suppression of HIV-transcribing cells. That study however did not investigate plasma HIV viremia nor demonstrate significant alterations in reservoir size in the subset of 13 participants assessed. We investigated the effects of COVID-19 mRNA vaccination on plasma viremia and reservoir size in 62 ART-treated individuals.

Methods: Samples were collected pre-vaccination, and at one month post-first and -second COVID-19 mRNA vaccine doses. HIV plasma viral loads (pVL) were measured using the Cobas 6800 system (lower limit of quantification = 20 copies/mL). Total and intact HIV copies/million CD4+ T cells were determined using the Intact Proviral DNA Assay (IPDA), using custom primers to accommodate within-host HIV polymorphism where needed.

Results: Of 62 participants (89% male), 43, 16 and 3 received BNT162b2/BNT162b2, mRNA-1273/mRNA-1273 or heterologous regimens, respectively. No significant changes in pVL were observed after vaccination (all $p > 0.2$, see Figure). HIV pVL was <20 copies/mL in 82% of samples pre-vaccination (range: <20–110 copies/mL), 79% one month post-first-dose (range: <20–183 copies/mL), and 85% one month post-second-dose (range: <20–57 copies/mL). To date, IPDA is complete for 46 (74%) participants at pre-vaccination and post-first-dose. No significant changes in intact reservoir size were observed between these timepoints: prior to vaccination, the median intact reservoir size was 77 (IQR:27–195) HIV copies/million CD4+ T-cells; one month post-first-dose, the median was 86 (IQR:36–194) ($p = 0.26$). The 5′ and 3′ defective proviral burdens also showed no temporal trends (all $p > 0.15$), and the lack of temporal trends in pVL and intact reservoir measures remained when stratifying the data by sex and vaccine regimen (all $p > 0.2$).

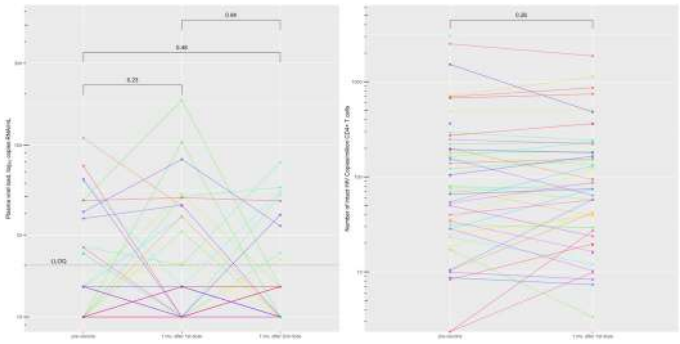

Figure. Plasma Viral Load (left) and IPDA (right) Results

Conclusions: While COVID-19 mRNA vaccines may stimulate HIV-specific immune responses in ART-treated individuals, our preliminary results suggest that they do not induce measurable changes in intact reservoir size nor induce lasting plasma HIV viremia.

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PP 1.38 – 00212

Characterization of SIV infected mast cells in early foci of rebound after ATI and HIV infection of primary mast cells in tissue culture models reveals an important new player in viral persistence and pathogenesis

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Background: HIV persistence during ART has traditionally been attributed to a population of long-lived, latency, infected memory T-cells. A PET/CT ^{64}Cu-FAB2 probe (7D3) used to localize SIV envelope expression, revealed SIV-infected mast cell (MCs) in the rebounding zone of expanding viral replication within mucosal and lymphatic tissues during the eclipse phase. The identification of these infected mast cells is consistent with multiple publications reporting HIV and SIV infection of MCs published in the 2000's. MCs are known for their role in allergy and anaphylaxis and coordinate the innate and adaptive immune systems by releasing key mediators of inflammation.

Methods: Viral replication was assessed by mCherry and GFP reporter expression. Additionally it was tracked by p24 expression after supernatant quantification. As previously reported, the infected MC culture exhibited a decreased p24 expession over time. LPS,or other NFkB stimulating treatment could reverse viral quiescence, stimulate p24 expression, and facilitate viral spread in tissue culture. Productive infection was confirmed by quantification of proviral DNA and immunofluorescence staining for Gag and envelope.

Results: Primary skin and mucosal MCs and MC derived cells lines were found to express CD4 and CCR5 in variable frequencies making them suspectable to R5-topic viruses. Thus considering their long-life and susceptibility to HIV infection, we investigated MC-derived cell lines and primary skin and gut MCs for their susceptibility to HIV infection. We find that MCs are readily infected by HIV and SIV. Viral replication was typically observed to be reduced over time, as mast cells return to their resting state. When p24 production decreased overtime, LPS stimulation could reverse viral quiescence, stimulating p24 production and viral spread in the culture.

Conclusions: Taken together, these data suggest MCs play a key role in SIV/HIV replication during infection. The central role of MCs as mediators of inflammation is consistent with a role in chronic inflammation, even during efficient viral suppression. The observed ability of viral production in infected MCs to become quiescent as the MCs returns to its normal resting state, suggests that normal resting MCs cells could contribute to residual viremia while on ART, and furthermore could re-seed HIV rebound of infection during ATI.

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PP 1.39 – 00086

The level of cell activation is associated with the pre-integrative latency of HIV linear DNA

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Background: Unintegrated HIV DNA represents between 20 to 35% of the total viral DNA in infected patients. Only the unintegrated linear DNA (ULDs) can be substrates for integration and for the completion of a full viral cycle. In quiescent cells, these ULDs may be responsible for pre-integrative latency. The objective of our study was to determine the dynamics and the half-life of ULDs depending on the activation of the host cell.

Methods: Due to the lack of specificity and sensitivity of existing techniques, the detection of these ULDs remains difficult to date. We developed an ultra-sensitive, specific and high-throughput technology for ULD quantification called DUSQ (DNA Ultra-Sensitive Quantification) which combines linker-mediated PCR with NGS using molecular barcodes. We then used the DUSQ technology to calculate the half-life of ULDs in activated or quiescent T-cells and in macrophages, in the absence of integration.

Results: In activated T-cells, we were able to determine that the ULD half-life was of 0.5 days (allowing a persistence of about 5 days). In contrast, in macrophages, the half-life was of 3 days (i.e. a persistence of about 1 month) and in resting T-cells it was up to 11 days (i.e. a persistence of 3 months). Finally, as a proof of concept of the use of DUSQ in human cohorts, we were able to quantify ULDs in spleen and blood samples from patients chronically infected with HIV-1.

Conclusions: Using the DUSQ technology, our results estimate for the first time the half-life of ULDs in cells as a function of their activation level. Indeed, in the absence of integration, pre-integration latency exists primarily through the cells' ability to degrade or circularize ULDs. These results provide proof of concept for the use of DUSQ in future cohorts to track pre-integrative latency. Moreover, DUSQ can be easily adapted to the detection of any other rare DNA molecules that are not easily quantifiable by existing molecular biology techniques.

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PP 1.40 – 00074

Blood Brain Barrier Pericytes and the Molecular Impact of Active and Latent HIV Infection

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Background/Aims: One of the prevailing health concerns in HIV-1 management is that virally suppressed patients remain at increased risk of HIV-associated comorbidities. Indeed, several epidemiological studies have delineated a higher susceptibility to mental health and neurodegenerative diseases where 15–20% suffer from depression, 1–5% experience a stroke in their lifetime, and 4–34% show ischemic lesions at autopsy. The CNS was once thought to be protected from infection however recent experiments on cells of the neurovascular unit show that astrocytes, microglia, and blood brain barrier pericytes are all capable of active and latent HIV infection. BBB pericytes are multifunctional cells that regulate brain paracellular and transendothelial fluid transport, maintain homeostasis of the CNS microenvironment, and maintain BBB integrity. Due to their position between the periphery and the CNS we hypothesize that BBB pericytes are a key cell type for understanding the neuropathologies experienced by patients with HIV.

Methods: We use a novel HIV reporter, named HIVGKO, a dual fluorescent reporter that allows for identification of cells at different stages in HIV infection. The molecular signatures of actively infected pericytes and their latently infected counterparts within the same culture were assessed using fluorescence cell sorting, RNA isolation, and RNA sequencing.

Results: Statistical analysis reveals several distinct molecular signatures when comparing latent, active, and uninfected cell populations. Among the most distinct pathways between latent and active infected pericytes are estrogen receptor signaling, mitochondrial dysfunction, and the sirtuin signaling pathway.

Conclusion: These findings provide key insights into the molecular signatures affecting HIV infection of BBB pericytes and may help to develop future targets for the treatment of HIV at the neurovascular unit.

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PP 1.41 – 00092

Spatial Transcriptomics and the Search for the Latent HIV-1 Cell Niche

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Background/Aims: Although ART decreases detectable levels of HIV-1 RNA in the peripheral blood, it does not eliminate latently infected reservoirs within tissues. Viral replication rebounds once treatment is interrupted and identifying these persistent HIV-1 reservoirs in virally suppressed patients presents a major roadblock to HIV-1 Cure research. Molecular characterization and manipulation of these persistent HIV-1 reservoirs within human peripheral lymph provide valuable information on the virological and immunological microenvironment that limits viral eradication. Human tissues may provide a more physiological model to bridge classic in vitro experiments to human HIV-1 Cure clinical trials.

Methods: We characterized the transcriptomic profile of persistent HIV-1 reservoirs in primary human lymph nodes using the 10X Visium platform. For spatial transcriptomics analysis, lymph nodes were snap frozen immediately following biopsy and embedded in OCT. Lymph node sections (10μm) were placed onto the 10X Visium slides, stained with H&E and imaged prior to RNA-Seq library prep.

Results: Following sequencing and bioinformatics analysis with 10X SpaceRanger software, data visualization was done using the 10X Loupe Browser and additional bioinformatics pipelines. We ran multiple analyses to increase our sample resolution and data yield. Harmony is an algorithm that groups cell by type and integrates data sets from multiple tissues, effectively eliminating variation between data sets to produce a single product. BayesSpace and SPOTlight resolve the issue of limited resolution of the spots on the 10X Visium platform. Finally, we used MISTy (Multiview Intercellular SpaTial modeling framework), which is a machine learning program that maps the cell-cell interactions and the relationships between the structural regions of the tissue. MISTy examines single cell specific information (intrinsic view), neighboring cells (juxtaview), and whole tissue information (paraview) to investigate specific tissue niche.

Conclusion: Molecular characterization and biomarker identification of persistent HIV-1 reservoirs that reside within human tissues has been a major hurdle in the fight against HIV-1. To address this, we analyzed human lymph nodes using the 10X Visium platform to spatially characterize the transcriptomic profile of persistent HIV-1 reservoirs in human lymph nodes.

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PP 1.42 – 00114

Functional Polarization of Human Monocyte-Derived Macrophage into M1-Proinflammatory Cells Restricts Both HIV-1 and Zika Virus Replication

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Background/Aims: M1-polarization by short-term stimulation with pro-inflammatory cytokines of human MDM leads to a partial restriction of R5 HIV-1 replication. Furthermore, restimulation of infected M1-MDM with the same cytokines several days after infection drives HIV-1 to a state of reversible latency. Therefore, we have investigated whether a similar pattern could be observed upon MDM infection by a different RNA virus, i.e. ZIKA virus (ZIKV), a flavivirus associated with an outbreak of microcephaly in newborns.

Methods: Human monocytes were purified by PBMC by Percoll density gradient and allowed to differentiate into MDM for 5–7 days. Cells were then incubated or not with IFN-g and TNF-a for 18 h in order to obtain M1-MDM. Cytokines were then removed and both M1 and control, unpolarized cells were infected either with R5 HIV-1 BaL or with different ZIKV strain at the MOI of 0.1 and 1, respectively. HIV-1 replication was

monitored by RT activity whereas ZIKV replication was quantified by PFU assay. Additional methods were adopted to further characterize the pattern of viral restriction in M1 cells observed with both human RNA viruses.

Results: M1-polarized MDM restricted R5 HIV-1 replication while it upregulated the expression of APOBEC3A (A3A), a putative restriction factor; restimulation of infected M1-polarized MDM with the same pro-inflammatory cytokines 7 days after infection drove virus expression into a quasi-latent state that was, however, reversible by coculture with allogenic PHA-stimulated PBMC, as reported. These findings were confirmed by in situ hybridization analysis for simultaneous HIV DNA and RNA detection (unpublished). Concerning ZIKV infection, this flavivirus productively infected both freshly isolated human monocytes and, even more efficiently, their MDM. M1-polarization significantly inhibited ZIKV replication in MDM. Neither monocytes nor MDM showed evidence of cytopathic ZIKV infection. Among putative entry receptors for ZIKV, Mer, but not Axl, was highly expressed by MDM and was strongly downregulated in M1 cells vs. control and M2-MDM. M1 polarization of human MDM resulted in the upregulation of ISG such as OAS2, MXA and ISG15.

Conclusion: M1 polarization of MDM leads to a partial restriction of multiple human RNA virus infection, as HIV-1 and ZIKV replication likely involving multiple mechanisms.

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PP 1.43 – 00206

Evaluating integrated HIV-1 quasispecies using Near Full Length sequencing in ART-suppressed individuals in the Drexel CARES Cohort

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Background/Aims: The integrated human immunodeficiency virus type 1 (HIV-1) provirus forms a stable but latent viral reservoir in various tissues. It has been shown that both replication competent and defective proviral sequences exist in the viral reservoir. At least some defective proviruses are able to produce viral proteins. While many individuals achieve undetectable levels of replicating virus as a result of anti-retroviral therapy (ART), current methods for monitoring viral load may not reflect the full landscape.

Methods: Droplet digital PCR (ddPCR), along with near full-length (NFL) amplification and third-generation sequencing (TGS) are valuable tools to evaluate the absence or presence of genes which may contribute to chronic HIV-1 neuropathogenesis and disease. Furthermore, these strategies can help predict and evaluate efficacy of CRISPR gene editing techniques. Sequencing the PBMC compartment has shown an approximate 30 percent failure of amplification of the viral genomic regions capable of encoding viral accessory proteins. We hypothesized that the reason for such differential results could be due to accumulation of replication incompetent defective proviruses. Given these observations, we have used NFL strategies to target provirus and examine the full spectrum of integrated proviruses within the PBMC compartment of individuals in context of cognitive impairment from the Drexel patient cohort and between the spleen and brain from the National NeuroAIDS Tissue Consortium (NNTC) Cohort.

Results: Amplicon analyses were conducted utilizing theMinION Nanopore TGS platform. Initial results showed ART-suppressed individuals had mostly defective virus as the predominant species with the number of full-length amplicons differing at the patient level with no clear clinical link.

Conclusion: Art-suppressed individuals have a majority of defective virus but full-length reads are still observed linking to why individuals rebound after ART-cessation. Future analyses will apply the NFL strategy to examine the potential role of defective genomes in the emergence of neuroHIV.

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PP 1.44 – 00107

Longitudinal evaluation of epigenetic age among people living with HIV (PLWH) undergoing multimodal curative interventions

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Background: Accelerated epigenetic aging is associated with HIV infection based on epigenetic clock algorithms constructed from dynamic DNA methylation states that change with age. We previously showed candidate host epigenetic marks predicative for HIV reservoir size, responsiveness to latency reversal, and viral rebound. Yet, whether epigenetic age in PLWH is impacted and potentially accelerated by multimodal antiretroviral and immune-based curative interventions remains undetermined.

Methods: Retrospective longitudinal DNA methylation profiles were obtained from 30 male PLWH on ART at baseline, week 24, and week 48 who participated in combination interventions designed for eradicating residual plasma viremia and decreasing HIV reservoirs (NCT02961829). Six study arms with 5 participants each included: 1. Continuation of ART, 2. Intensified ART (ART + Dolutegravir + Maraviroc), 3. Intensified ART and HDACi (ART + Dolutegravir + Maraviroc + Nicotinamide), 4. Intensified ART and Auranofin (ART + Dolutegravir + Maraviroc + Auranofin), 5. Partially intensified ART (ART + Dolutegravir) followed by dendritic cell vaccine, and 6. Partially intensified ART (ART + Dolutegravir) + Nicotinamide + Auranofin followed by dendritic cell vaccine. Group 3 included the São Paulo Patient that rebounded at 511 days following analytical treatment interruption.

Results: At baseline study entry prior to intervention for all participants, we observed that an increased DNA methylation inferred percentage of CD8 + CD28-CD45RA- T cells in blood significantly associated with shorter time to viral rebound after treatment interruption ($r = -0.44$, $P = 0.03$). We observed no significant group differences comparing baseline, week 24, and week 48 timepoints in epigenetic clock measures of biological age, mortality prediction, and telomere length (Horvath Clock, Hannum Clock, Horvath MultiTissue Clock, DunedinPoAm45, PhenoAge Clock, DNAmTL, and GrimAge Clock) for all six study arms. Notably, of all the participants assessed only the São Paulo participant exhibited a potential reversal of epigenetic age of ~15

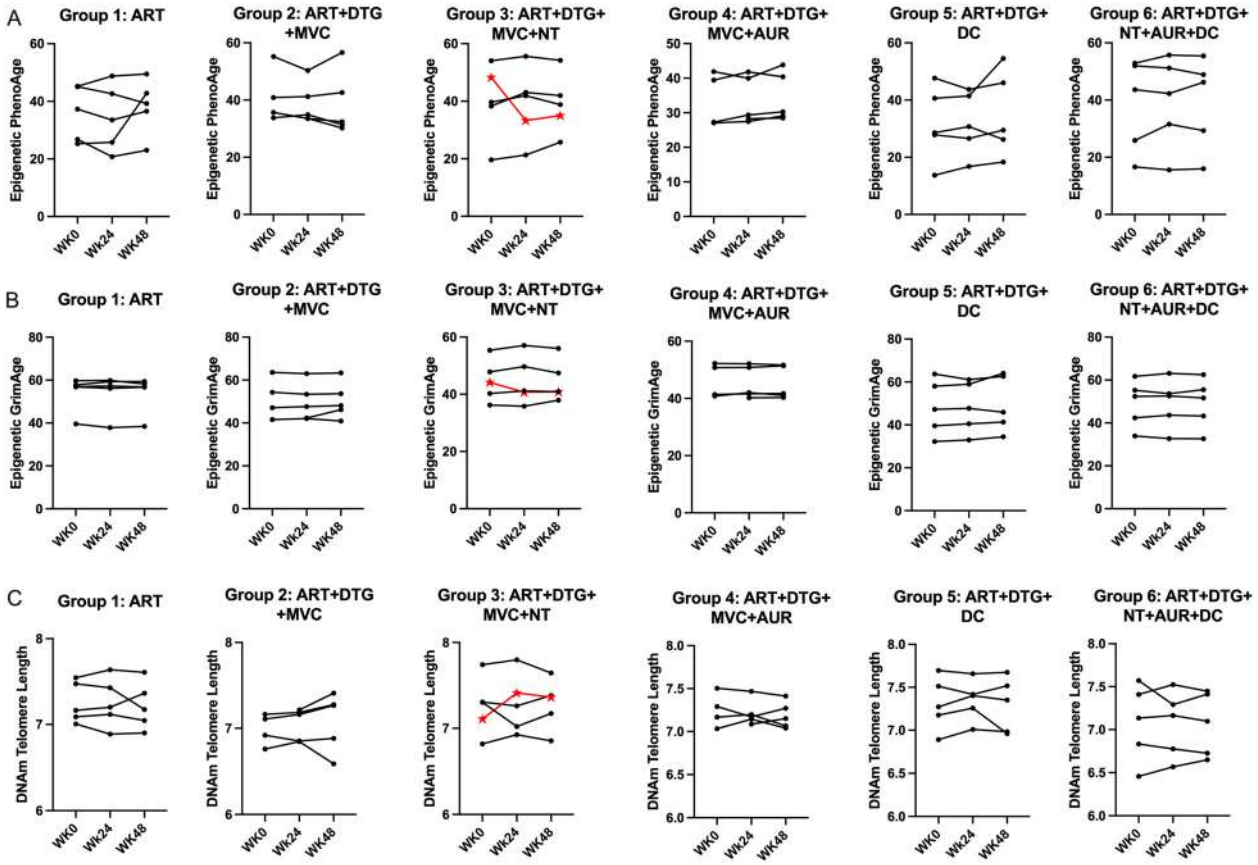

Figure 1 (abstract: PP 1.44–00107) Epigenetic clock estimates during cure trials.

years from an estimated epigenetic age of 42.8 years at baseline to 27.7 years at week 24 and 29.2 years at week 48. Additionally, this participant showed a decrease in epigenetic mortality risk and increased inferred telomere length.

Conclusions: Multimodal curative interventions in PLWH do not accelerate epigenetic biomarkers of aging, mortality risk, and pace of aging. Findings from the São Paulo patient suggest that potential interventions that lead to a reversal of epigenetic age may determine rebound delay.

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Session 2: In Vitro and Animal Model Studies of HIV Persistence

OP 2.1

CCR5 in HIV Prevention and Cure

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Background: CCR5 plays a critical role in HIV infection as the major viral co-receptor. Individuals naturally lacking surface CCR5 expression through a homozygous 32 base pair deletion ($CCR5^{\Delta32/\Delta32}$) are highly resistant to HIV infection, and the only documented cases of HIV cure occurred following allogeneic stem cell transplantation from $CCR5^{\Delta32/\Delta32}$ donors. Mimicking the $CCR5^{\Delta32/\Delta32}$ phenotype is therefore an attractive avenue for both HIV prevention and cure.

Methods: Leronlimab is a CCR5-specific monoclonal antibody that binds to the extracellular loop-2 and N-terminus domains of CCR5, thereby directly outcompeting HIV for CCR5 engagement and blocking entry to CD4+ T cells. We generated long-acting leronlimab and administered it to rhesus macaques prior to weekly rectal challenge with CCR5-tropic SHIV. To test use of leronlimab in functional cure, an AAV vector expressing long-acting leronlimab was administered to macaques with established SHIV infection.

Results: A single dose of long-acting Leronlimab achieved full CCR5 receptor occupancy (RO) on CD4+ T cells isolated from both peripheral blood and rectal biopsies for >12 weeks. A single dose of long-acting leronlimab also significantly protected against acquisition following repeated, low dose rectal challenge with CCR5-tropic SHIV. In macaques with established CCR5-tropic SHIV infection, resolution of plasma viremia occurred following the emergence of full CCR5 RO on CD4+ T cells in peripheral blood. Of note, we observed viral blips concomitant with transient loss of full CCR5 RO on CD4+ T cells in blood.

Conclusions: Given the safety and protective efficacy of the naturally occurring $CCR5^{\Delta32/\Delta32}$ phenotype, leronlimab is an attractive addition to the growing arsenal of long-acting injectables for HIV prevention. AAV vectored delivery of leronlimab demonstrated proof of concept that the $CCR5^{\Delta32/\Delta32}$ phenotype can be phenocopied by gene therapy delivery of CCR5 blockade.

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OP 2.2 – 00200

Characterization of the SIV tissue reservoir transcriptional environment at the single focus level during ART and post ATI

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Background: Despite effective antiretroviral therapy (ART), HIV-1 persistence is the major obstacle to cure. To eliminate this reservoir, it is key to identify the cell types involved in viral persistence and rebound and characterize their tissue micro-environment during ART, and at and after analytical treatment interruption (ATI).

Methods: We performed transcriptome analysis of tissues harboring SIV reservoirs on 20 RNA samples extracted from 10 to 20 um tissue sections from SIV-infected Rhesus macaques (RM). RMs were intravaginally challenged with SIVmac239, ART initiated at 4 days and maintained for 6 months, followed by PET/CT-guided necropsy either during ART or after ATI (early ATI: 4–10 days; late ATI: 18–24 days post-ATI). 64copper-labelled probe against viral envelope efficiently detected infection sites as early as four days post-ATI. Bulk RNA-seq analysis was performed on ART, early- and late ATI samples. In parallel, we optimized a pipeline for immunoPET/CT-guided spatial transcriptomics of tissue areas from tissues with detected SIV from same animals. Using the 10X-Visium platform and our own optimized pipeline, we characterized the transcriptomic environment of PET/CT+ "hot" areas of the reservoir vs non-reservoir areas in tissues.

Results: Differential expression analysis of the RNA-seq data indicates activation of viral responses and cell migration in tissues that harbor the reservoir two weeks after ATI. Instead, on ART tissue reservoirs were characterized by an environment in which catabolic processes and drug metabolism are upregulated, indicating ART effect on the tissues. Our newly developed immunoPET/CT-guided spatial transcriptomics allowed us to identify the transcriptional status of viral production foci of the tissue reservoir. In day18 post-ATI tissues with detectable SIV we identify foci of viral rebound associated to differential transcriptional patterns compared to non-reservoir areas. Here, we detect the activation of genes associated to metabolic processes, cell localization, and cytokine immune signaling. The combination of both analyses indicates that active SIV rebounding foci might be characterized by immune cell migration and cytokine signaling.

Conclusions: Our newly developed techniques combining an immunoPET/CT guided system with genomics and spatial transcriptomics allow us to identify with unprecedented detail possible markers of tissue reservoirs and characterize the cell types involved in maintenance and recovery of the SIV reservoir.

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OP 2.3 – 00117

The EZH2 inhibitor Tazemetostat increases MHC I antigen presentation in vitro and in vivo, enhancing antiviral activities of HIV-specific CTLs

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Background: Target cells are active partners in cytotoxic T-lymphocyte (CTL) mediated killing and vary in their intrinsic susceptibilities to this. We reported that overexpression of BCL-2 in reservoir-harboring cells confers a degree of resistance to CTLs, and that a BCL-2 antagonist potentiated 'kick and kill' reservoir reductions ex vivo. Here, we evaluate overexpression of Enhancer of Zeste Homolog 2 (EZH2) as a putative additional mechanism of resistance, implicated by its transcriptional over-expression in HIV-infected $CD4^+$ T cells that survive CTL co-culture. EZH2 is a histone-methyltransferase that negatively regulates expression of MHC-I and is inhibited by the FDA-approved drug Tazemetostat.

Methods: Cultures of primary HIV-infected $CD4^+$ T cells were treated with Tazemetostat for 5 days and incubated overnight with HIV-specific CTLs. Specific killing was evaluated by quantifying changes in viable infected cells by flow cytometry. Nod SCID-/-IL2rgnull mice were engrafted with human memory $CD4^+$ T cells isolated from an HIV+ individual. After 5 weeks, mice were infected with HIV_{JRCSF} and co-engrafted with autologous memory $CD8^+$ T cells or maintained as no-CD8 controls. Tazemetostat or vehicle control were administered orally, up to 500 mg/kg BID. HIV viral loads and phenotypical analysis of T cells were conducted weekly.

Results: Co-culture of infected cells with HIV-specific CTL clones (at effector to target ratio of 1:2) resulted in elimination of 50–70% of infected cells. Pre-treatment with Tazemetostat induced higher MHC-I levels on infected cells and a significantly higher degree of killing (up to 80–90%). No such enhancement was observed with cells infected with a Nef-deficient virus, suggesting that Tazemetostat may act by offsetting Nef-mediated MHC-I downregulation. In vivo, viral loads were significantly reduced in +CD8 versus no-CD8 mice. Tazemetostat drove a 2.1-fold increase in surface MHC-I on infected cells ($n = 8$, p value = 0.0004), and a further decrease of 0.56-log (mean value) of viral load, relative to vehicle control +CD8 mice.

Conclusions: Tazemetostat increases basal MHC-I expression in $CD4^+$ T-cells, counterbalancing Nef-mediated immunoevasion. This results in enhanced infected-cell elimination in vitro

and decreased viral loads in vivo. These results provide impetus for pre-clinical studies assessing impact on reservoir formation and for consideration of clinical studies with this FDA-approved compound.

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OP 2.4 – 00145

No Evidence of Ongoing Viral Replication in SIV-Infected Macaques on Combination Antiretroviral Therapy Initiated in the Chronic Phase of Infection Despite Elevated Residual Plasma Viral Loads

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Background: SIV-infected macaques on combination antiretroviral therapy (cART) often have higher residual plasma viral loads (PVLs) than HIV infected people on cART, suggesting ongoing viral replication during cART may be more readily detectable in this model, if it occurs. Here, we sought evidence of ongoing viral replication by using cART intensification and state-of-the-art viral quantification and viral genome sequencing analyses in SIV-infected rhesus macaques that initiated cART during chronic infection.

Methods: Ten Indian-origin rhesus macaques were treated with cART (TDF/FTC/DTG) for >2 years starting 8 weeks after IV infection with the barcoded virus SIVmac239M. In five animals (Group 1), the first 24 weeks of treatment were intensified with the protease inhibitor darunavir (DRV), which is active against SIV in macaques. DRV was then discontinued in Group 1 and added to Group 2 for another 12 weeks. PVL, cell-associated viral RNA (CA-vRNA), DNA (CA-vDNA), and 2-LTR circle DNA were quantified by qRT-PCR and qPCR. Longitudinal single-genome sequencing of env was performed for study animals on cART and untreated control animals with comparable PVL area-under-curve (AUC) to compare viral evolution over time.

Results: Animals were assigned to groups to balance their pretreatment PVLs (range 3.0 × 104–6.2 × 107 vRNA copies/ml). Regimen intensification had no impact on initial PVL decay kinetics, PVL AUC prior to and following DRV crossover, CA-vDNA, CA-vRNA:CA-vDNA ratio, or 2-LTR circle levels. Time to suppression to <15 vRNA copies/ml plasma was associated with pretreatment PVL irrespective of treatment intensification. Viral sequence evolution for animals on cART with elevated residual PVLs (>102 vRNA copies/ml) was compared with untreated controls. Sequence diversity, divergence, slope of p-distance over time and panmixia tests revealed no evidence for viral evolution in animals on cART compared to untreated controls.

Conclusions: We found no evidence of ongoing viral replication in SIVmac239M infected animals on cART, suggesting that residual viremia is attributable to viral production from already-infected cells that persist during cART. These results highlight the relevance of NHP models of cART mediated virologic suppression and suggest that ongoing viral replication is unlikely or may be difficult to detect in cART suppressed HIV-infected people.

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OP 2.5 – 00048

Targeting the SIV reservoir with Alemtuzumab

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Background: Alemtuzumab (AZM) is a licensed pan-lymphocyte-depleting monoclonal antibody (mAb) that targets CD52+ cells. Here we administered AZM to SIV-infected rhesus macaques (RM) at the time of ART initiation (early) or during full ART suppression (late) to assess its ability to deplete latent, SIV-infected cells.

Methods: RM were initially screened for CD52 expression on erythrocytes and then IV inoculated with 500 infectious units of the barcoded SIVmac239M. ART was initiated 7 days post-infection (dpi). RM also received 4 weekly doses of AZM or control mAb at 5 mg/kg starting 7 dpi (early; n = 8 AZM, n = 6 control) or 294 dpi (late; n = 8 AZM, n = 4 control). ART was discontinued at 533 dpi to assess SIV rebound dynamics.

Results: AZM induced massive lymphocyte depletion, including >95% of CD4+ T cells in blood. CD4+ T cell depletion was also observed in the lymph nodes. Depletion was followed by CD4+ memory T cell proliferation and the reconstitution of cells in blood. Following ART cessation, 24 of 26 RM rebounded, with no difference in time to rebound or rate of reactivation of SIVmac239 barcodes between AZM-treated RM and controls. However, time to rebound did correlate with pre-ART peak pvl. Interestingly, 2 early AZM-treated RM with lowest pvl at time of ART showed no rebound >400 days post-ART release.

Conclusions: Despite depletion of circulating CD4 memory T cells, Alemtuzumab treatment was insufficient to delay or prevent SIV rebound in most RM. However, the lack of rebound in 2 of 8 early AZM-treated RM suggests the reservoir may be more labile at the time of ART initiation.

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OP 2.6 – 00135

Constitutive NKG2A levels and timing of antiretroviral therapy initiation impact the potential role of NK cells after treatment interruption - the pVISCONTI study

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Background: The pVISCONTI study aims to assess the mechanisms leading to post antiretroviral treatment (ART) control in a cynomolgus macaque (CyM) model of SIVmac251 infection. We found that a 24-month ART initiated at day 28 post infection (p.i.) favored a delayed viral rebound after treatment interruption (TI) and a high frequency of post-treatment controllers when compared to ART initiated at 6 months p.i. We evaluated the impact of time to ART initiation on NK cell dynamics, and their potential role on post treatment SIV control.

Methods: SIVmac251-infected CyMs initiated ART at early (28 days p.i., n = 12) or late phase of infection (6 months (p.i.), n = 12) and continued ART for 24 months. The treatment was interrupted and the animals were monitored for 6 to 12 months. We analyzed phenotypically (flow cytometry) NK cells from blood and tissues (Lymph Nodes (LN), Bone Marrow (BM) and Broncho Alveolar Lavage (BAL)) at various timepoints before ART and following ART interruption (TI).

Results: We found that NK cells from CyMs constitutively expressed different levels of NKG2A in blood, LN and BM. The presence of NK cells with low expression of NKG2A before infection was significantly correlated with a higher viral load during primary-infection. In addition, unsupervised analysis of NK cell subsets at the baseline revealed that NKG2AlowNKp46high NK cells positively correlated with viral load during the first days following TI. The timing of the treatment initiation had a strong impact on the phenotype of the NK cells. NK cells from the late-treated group showed a distinct phenotype from the early- treated group after two years under ART and these differences increased after TI. A higher frequency of NKp46 + NKp30 + NK cells was observed in late-treated animals from day 3 after TI and was associated with a higher viral load at day 28 after TI.

Conclusions: The study suggests that the level of constitutive expression of NKG2A on NK cells is a marker for the capacity to control SIV viremia during primary-infection. We also show that the timing of ART impacts the distribution of the NK cell subsets and their mobilization after TI.

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OP 2.7 – 00057

The latency reversing agent HODHBt synergizes with IL-15 to enhance cytotoxic function of HIV-specific CD8 + T-cells

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Background: IL-15 is being investigated in multiple pre-clinical and clinical "Kick and kill" studies, but increased potency through combination with other agents may be needed to achieve reductions in HIV reservoirs. 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (HODHBt) enhances IL-15-mediated HIV reactivation in cells from people living with HIV, by increasing STAT5 occupancy on the HIV-LTR. Since IL-15 also acts on CD8 + T-cells through STAT5 activation, we hypothesized that HODHBt would synergize with IL-15 to enhance HIV-specific cytotoxic T-cell responses.

Methods: PBMCs from 3 ART-treated participants were treated with IL-15 or IL-15 + HODHBt and subjected to scRNAseq. GZMB ELISpots were performed on PBMCs from 14 participants - suppressed on ART for an average of 10.9 years - stimulating with peptide pools spanning Gag, Pol, Nef, Env, or CMV-pp65, with or without IL-15 and HODHBt. Secreted cytokines were measured in supernatants by CorPlex. HIV eradication assays (PBMC-HIVEs) were performed; briefly: ex-vivo PBMCs from 3 ART-treated participants were treated with single agent or combination IL-15 and HODHBt, with NK, or NK & CD8 depletion or complete PBMCS for 9 days. CD4 + T-cells were isolated and subjected to the Intact Proviral DNA Assay. At days 3 and 9, cells were phenotyped by flow cytometry and p24 release was measured by ultrasensitive ELISA.

Results: HIV-specific GZMB-releasing responses were enhanced by treatment with IL-15 + HODHBt relative to IL-15 + DMSO, as follows (medians): Gag 4.4-fold ($p < 0.002$), Pol 2.4-fold ($p < 0.020$), and Nef 27.8-fold ($p < 0.001$). GZMB responses correlated with soluble IFNγ ($r = 0.89$, $p < 0.001$) and IL-22 ($r = 0.73$, $p < 0.001$). scRNAseq data shows T-cells upregulated antigen processing and presentation genes in response to IL15 + HODHBt, and CD4 + T-cells upregulated surface MHC-I. PBMC-HIVEs showed increases in T-cell activation (CD69), pSTAT5 expression, and levels of perforin and granzymes in the IL-15 + HODHBt conditions in all 3 donors. Intact HIV DNA was significantly reduced in 1 of 3 donors treated with IL-15 + HODHBt, in both whole PBMCs ($p = 0.029$)

and NK-cell depleted conditions ($p = 0.032$), but not in the NK-cell + CD8-depleted condition ($p = 0.692$).

Conclusions: Pharmacologic enhancement of IL-15 mediated STAT5 activation potently enhances cytotoxic HIV-specific CD8+ T-cell responses. Together with known latency reversal activity, this can be sufficient to drive ex vivo HIV reservoir reductions, though this appears to vary by donor.

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OP 2.8 – 00193

Enhancing PKC Modulator HIV Latency Reversing Agents

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Background: HIV persists during antiretroviral therapy (ART) in latently infected CD4+ T cells. One possible strategy for eliminating these reservoir cells is to induce expression of the latent provirus using latency reversing agents (LRAs), allowing the host cell to be killed by the immune response or viral cytopathic effects. While no class of LRA has thus far been demonstrated to induce expression of all latent HIV in vivo, several LRA classes have shown promise using in vivo models and are thus candidates for the development of improved LRAs. Here, we describe the design, development, and in vitro and in vivo characterization of new latency reversing agents that function through the PKC pathway.

Methods: New PKC modulators based on bryostatin, prostratin, or ingenol scaffolds were designed and synthesized. These include fully active analogs and "slow-release" prodrug compounds, which are designed to become slowly active over time in vivo to improve biodistribution and tolerability. Compounds were evaluated in vitro using cell lines, ex vivo using primary cells, and in vivo in humanized bone marrow-liver-thymus (BLT mice). In vivo studies involved the use of a barcoded HIV strain that allows more accurate quantification of rebound-competent HIV reservoirs that emerge after stopping ART.

Results: In vitro analysis identified several synthetic analogs of naturally occurring PKC modulators which out-performed the parent compounds that inspired their design. Prodrug PKC modulators also showed delayed activity compared to fully active compounds as intended. Importantly, several prodrugs were better tolerated in mice compared with the fully active compounds, in some cases increasing the range of tolerated bioactive doses by over 20-fold. Finally, when a synthetic bryostatin analog was administered to infected humanized mice during ART it delayed rebound after stopping ART and reduced the genetic diversity of rebounding virus, indicating a reduction in rebound-competent reservoirs.

Conclusions: These data show that PKC modulator LRAs can be designed and synthesized which are better tolerated and more effective than the naturally available compounds. This class of LRA might therefore prove useful in future combinatorial "kick and kill" approaches for HIV cure.

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PP 2.1 – 00014

Impact of early antiretroviral therapy on tissue resident myeloid cells in the liver and lung of SIV-infected rhesus macaques

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Background: Viral dissemination occurs early after infection, targeting CD4 T cells and monocytes/macrophages. Monocytes derived from bone marrow and tissue resident macrophages (TRMs) derived from yolk sac, are short-lived and long-lived cells, respectively. Whereas we recently demonstrated that early antiretroviral therapy (ART) efficiently prevents the infection of monocytes in SIV-infected rhesus macaques (RMs), little is known so far about TRMs, and whether these cells may represent viral reservoirs. Herein, we assessed the impact of early antiretroviral therapy on tissue resident myeloid cells in the liver and lung of SIV-infected rhesus.

Methods: RMs were infected with SIVmac251 and treated at day 4 post-infection with a cocktail of antiretroviral drugs. Cells from liver and lung were mechanically isolated. The phenotype of TRMs was analyzed by flow cytometry using specific monoclonal antibodies directed against the CD14, CD16, CD44, TIM-4, CD117, CD206, CD200R, CD64 and LYVE-1 molecules (previously defined in mice). The levels of viral DNA and RNA were quantified by qPCR. In situ hybridization was used to detect vRNA. Furthermore, transcriptomic analysis was performed to assess gene profiles in both tissues.

Results: Our results revealed that CD117, CD206 and LYVE are specific markers of TRM cells from liver and lung of SIV-infected RMs. In non-treated SIV-infected RMs, higher levels of inflammatory and ISG (*interferon stimulated genes*) transcripts were observed consistent with the detection of viral RNA and DNA in both tissues. The levels of cell-associated viral RNA are positively correlated with plasma viremia. By sorting myeloid cells, we demonstrated that circulating monocytes and TRMs from liver and lung both contained viral DNA in non-treated individuals. Furthermore, early ART, which prevented monocyte infection, also prevented the establishment of viral dissemination in both tissues as well as the inflammation.

Conclusions: Herein, we analyzed the phenotypes of TRMs in the lung and liver of SIV-infected RMs. We demonstrated that early ART efficiently prevents viral seeding both in the liver and lung concomitantly by preventing inflammation. These results highlight the crucial importance of early treatment by decreasing anatomical viral reservoirs, thus improving the health of people living with HIV.

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PP 2.2 – 00024

Evaluation of an NNRTI-mediated Targeted Activated Cell Kill (TACK) in a Viremic Mouse Model

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Background: Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a validated class of antiretroviral molecules that bind to and inhibit HIV-1 reverse transcriptase (RT). In addition to the RT inhibition, certain NNRTIs can cause cytoplasmic

dimerization of gag-pol leading to HIV protease activation and a targeted activated cell kill (TACK) effect specific to infected cells.

Methods: Here we evaluate the TACK mechanism in a viremic, humanized mouse model of HIV-1 infection. The compound used in this work is QZ06 which has shown in vitro to be a NNRTI with potent RT inhibition and TACK activity. Viral decay within the mouse model was evaluated over time.

Results: In this mouse model, QZ06 suppresses plasma viremia at a faster rate than an integrase inhibitor drug (raltegravir) or an NNRTI (Pyr65) compound that lacks TACK activity. We hypothesize that the faster decay in viral burden with QZ06 is due to the HIV-1 infected cell kill activity eliminating virus-producing cells instead of only blocking further rounds of replication. To evaluate the TACK activity further, several biomarker assays were used to evaluate HIV RNA and HIV gag p24 levels in spleen.

Conclusions: Use of this viremic mouse model, together with these different biomarker modalities, will allow for further assessment of the effects of TACK active molecules compared to current HIV therapies.

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PP 2.3 – 00034

CD8+ T-Cell Sieving During SIV Reactivation from Latency

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Background: Immune escape from antibody and T-cell recognition is often observed in the HIV reservoir as well as in the replicating viral population after treatment interruption. However, whether escape mutant virus is preferentially selected during reactivation ('sieving' of reactivating virus) or whether escape variants arise due to mutation and immune selection after viral rebound from latency is unclear. In this study, we sought to identify the role of sieving and post-rebound immune selection in an SIV model of infection and latency.

Methods: 14 Mamu-A*01 rhesus macaques were infected with the barcoded SIVmac239M virus and initiated ART on day 14 post-infection. After 7 months of ART, animals underwent a 2-month analytical treatment interruption (ATI) before re-initiating ART. Finally, animals underwent a 2nd ATI after 3 months of ART, followed by CD8 depletion after 3 months of ATI in 10 animals. High throughput Illumina sequencing of the barcode and Tat-SL8 epitope (a known locus of early CD8 pressure in Mamu-A*01 rhesus macaques infected with SIV) was performed, and $CD8^+$ T-cell specificity was characterized by tetramer staining.

Results: Plasma virus was predominantly wild-type at the Tat-SL8 epitope (WT) during primary infection and early after rebound following the 1st ATI (mean 83% WT). Escape mutant virus became dominant by the end of the 1st ATI, (mean 15% WT). Early rebounding plasma virus after the 2nd ATI was predominantly escaped (mean 4% WT). Escape variants and viral barcodes present early after the 2nd ATI were related to the those seen in the same animal at the end of the 1st ATI, suggesting reactivation of escape mutant virus and suppression of WT virus. By contrast, barcodes newly emerging following the subsequent CD8 depletion had last been detected earlier in infection ($p < 10^{-7}$) and were more associated with WT at last detection ($p = 10^{-4}$), suggesting CD8-depletion allowed the reactivation of older WT virus. Surprisingly, despite the strong evidence for sieving during the 2nd ATI, Tat-SL8-specific $CD8^+$ T-cells were only marginally higher at this time.

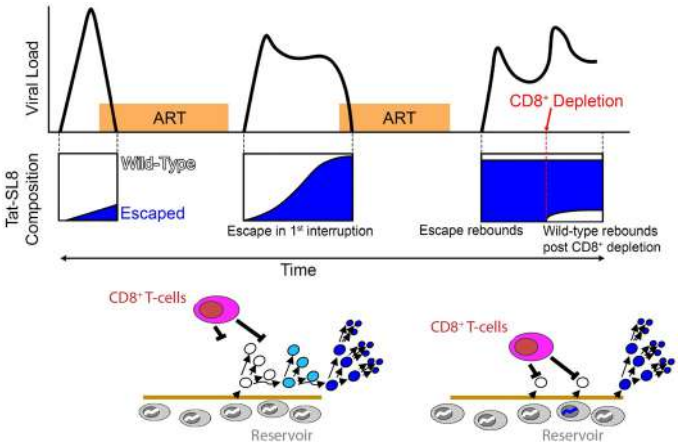

Figure. CD8 sieving of reactivating virus

Conclusions: CD8+ T-cells can act to block the reactivation of targeted SIV variants following ATI.

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PP 2.4 – 00038

Age and biological sex but not sex hormones influence IL-15 biological activity

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Background: The role of different biological variables including sex hormones, age, and biological sex in HIV cure approaches is not well understood. Previous studies have shown differences in HIV infection by biological sex, as well as differences from sex hormones in infection and transcription. The γc-cytokine IL-15 is a clinically relevant LRA that mediates HIV reactivation and promotes immune activation. A recent phase I clinical trial showed the safety and efficacy of the IL-15 superagonist N-803 in people living with HIV. In this work, we examine the interplay that biological sex, age, and sex hormones estradiol, progesterone, and testosterone may have on the biological activity of IL-15.

Methods: To evaluate effects on latency reversal, we utilized a previously established primary cell model of latency using 8 sex

and age-matched donors. To evaluate effects on immune activation, 20 HIV-negative, age-matched donors were used to measure activation of CD4 T, CD8 T, and NK cells and to measure cytokine secretion using a 10-cytokine array panel. Treatments of estradiol, progesterone, or danazol (a testosterone derivative) were used at three physiological concentrations, reflecting levels found in plasma during the life span. Finally, expression of hormone receptors was measured by western blot and flow cytometry.

Results: Sex hormones, age, and biological sex did not influence the ability of IL-15 to reactivate latent HIV in the primary cell model of latency. Moreover, none of the sex hormones tested influenced the ability of IL-15 to promote immune activation or cytokine secretion. Interestingly, IL-15 promoted higher CD4 T cell immune activation from female donors than from male donors (Mann-Whitney test, $p = 0.029$). Additionally, there was a positive correlation between CD8 T cell activation and age (spearman correlation, $r = 0.479$, $p = 0.033$). Finally, CD4 T, CD8 T, and NK cells expressed detectable levels of estrogen receptor (ER)-b, androgen receptor, and variable expression of progesterone receptor isoforms but lack detectable protein expression of ER-a.

Conclusions: Our study has found that biological sex and age, but not sex hormones, influence the biological activity of IL-15. Understanding how different biological variables affect the biological activity of cure therapies will help us evaluate current and future clinical trials aimed towards HIV cure in diverse populations.

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PP 2.5 – 00041

HIV persistence and latency in microglia: Single-cell transcriptome analysis of three humanized mice models of HAND shows viral responses to inflammatory signaling

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Background: It has been challenging to develop physiologically relevant human model systems to study HIV-associated neurocognitive disorder (HAND). HIV-infected human microglia from multiple humanized mouse backgrounds become latently infected but can become reactivated in response to inflammatory signals.

Methods: We compared microglia populations obtained from reconstituted JAX NSG CD34+, NOG-IL34, and MISTRG humanized mice. Mice were either infected with HIV with or without ART or uninfected. Brain glia fractions were isolated and analyzed using the BD Rhapsody scRNAseq platform using a panel of 41 antibodies to human immune cells and microglia surface markers to characterize key surface markers. Integrated transcriptome/surface proteome datasets were analyzed for differentially expressed (DE) genes and pathways using Seurat, Connectome, and SCENIC packages for R and Python. Trajectories of differentiating cells in pseudotime were elucidated using Monocle3, Velocyto, and Slingshot packages for R.

Results: Human microglia and other myeloid lineage cells represented up to 5% of the total glia. We observed a gradual differentiation of human monocytic lineage cells into CD209+/CD163+/P2RY14+ pre-microglial/perivascular cells to P2RY12+/TMEM119+/ITGAXhigh mature microglia, the latter being especially evident in the NOG-IL34 mice. Microglia

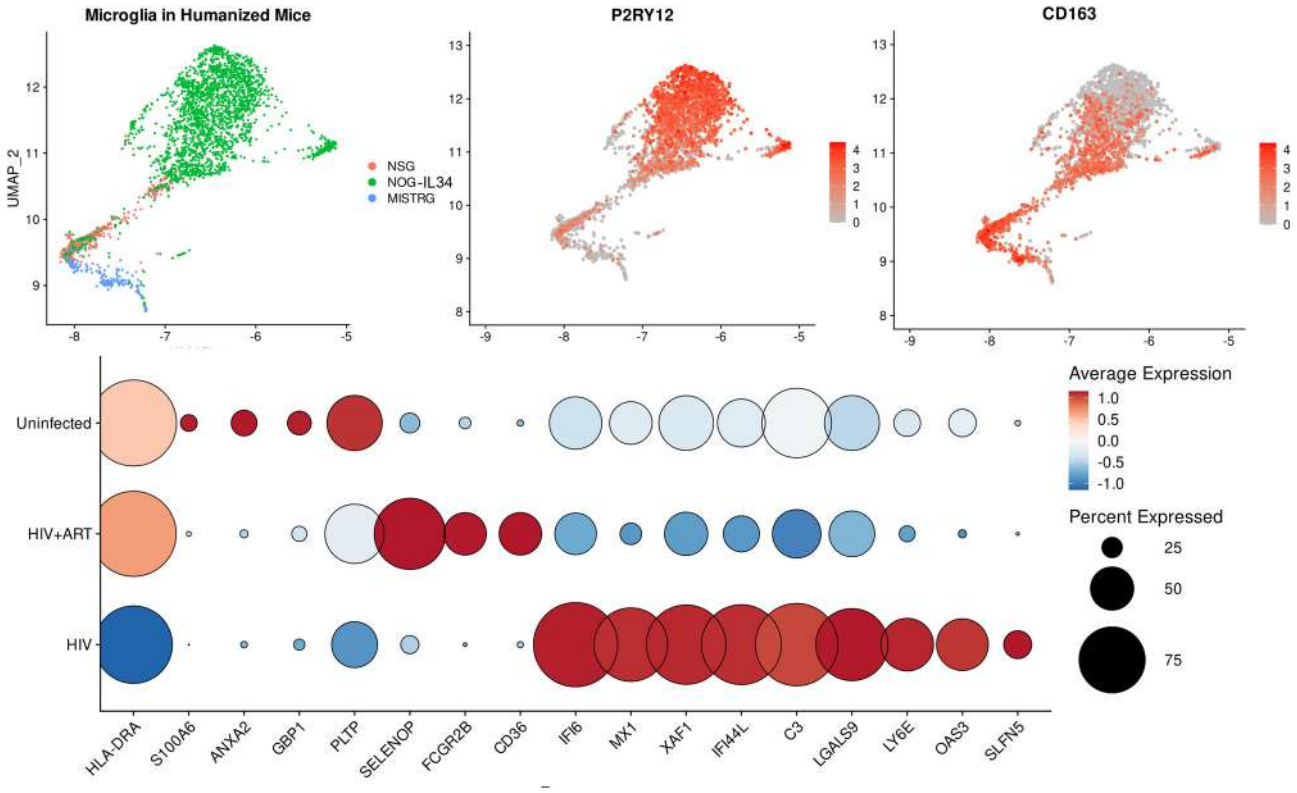

Figure 1 (abstract: PP 2.5–00041) Top markers in human microglia from humanized mice

from HIV-infected mice expressed multiple interferon- and viral-response genes (IFI6, IFI44L, MX1), as well as complement (C3) and other HIV-associated host genes (LY6E, LGALS9, SLFN5). All these genes were suppressed in the ART-treated mice.

Conclusions: Human microglial cells that were indistinguishable from the human brain-derived microglia can be recovered from humanized mice, but the yield of microglia and the proportion of mature microglial cells differed substantially between the various models. Productive HIV infection of microglial cells was seen in reactive microglia, whereas homeostatic microglia displayed minimal HIV transcripts. The ability of human microglial in the context of humanized mice to display innate immune signatures and respond to pro-inflammatory cytokines makes them a promising model to study the molecular and cellular mechanisms of HAND.

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PP 2.6 – 00043

Probing cell death pathways in response to NNRTI treatment using a THP-1 infection model

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Background: Certain non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as Efavirenz, have been reported to induce HIV-1 infected cell death through CARD8-mediated pyroptosis. We refer to this activity as targeted activator of cell killing (TACK). Probing the pathway which leads to the TACK effect can be challenging in primary cells due difficulty in achieving high infection rates and poor CRISPR editing efficiency to create clonal populations. A model that readily allows editing of the host cell's genome as well as higher infection rates can help aid in the pathway's study.

Methods: We have developed an infection model using THP-1 cells to further interrogate the TACK effect. This monocyte-like cell line can be readily infected by VSV-G pseudotyped HIV-1 with high efficiency and HIV-1 infected cells from this line are sensitive to the TACK effect of NNRTIs. Using CRISPR knockout methods, clonal lines were prepared with knockouts of various members of the apoptotic and pyroptotic pathways. The KO cells were then used to test the ability of TACK-active NNRTIs to selectively induce cell death of HIV-1 infected cells.

Results: Using these tools, we evaluated NNRTIs for their ability to specifically induce death in HIV-1 infected wild type cells as well as cells knocked out for members of the apoptotic and pyroptotic pathways. By identification of factors which are involved in NNRTI-induced cell death as well as factors which are not, we're able to gain a more complete mechanistic understanding of the TACK effect.

Conclusions: We describe here a THP-1 based model for studying TACK-active NNRTIs to further mechanistic understanding of NNRTI-mediated killing of HIV-1 infected cells. Since THP-1 cells are readily manipulated genetically, this allows for effective knock out and clonal selection of members of pyroptotic and apoptotic pathways to better tease out the exact mechanism of action of these molecules' infected cell killing ability. Additionally, this model could serve as an important screening tool to identify and unravel mechanism of action of novel agents capable of inducing HIV-1 infected cell death.

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PP 2.7 – 00093

Modeling HIV-1 Pathogenesis and Latency in iPSC-Derived Human Cerebral Organoids

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Background: Identifying and characterizing HIV-1 reservoirs within the central nervous system (CNS) in virally suppressed patients presents a major roadblock to HIV Cure research. The complex microenvironment of HIV-1 reservoirs within the CNS affects viral replication and antiretroviral efficacy. Additionally, HIV-1 associated neurocognitive disorders (HAND) may develop in people living with HIV-1, even while taking ART. Understanding HAND development and progression is critical to improve HIV-1 treatments and may help advance cure strategies.

Methods: To model HIV-1 infection and latency in human CNS tissue, we modified a human induced-pluripotent stem cell (hi-PSC) derived cerebral organoid model developed by the Fine lab to include monocytes and microglia. These organoids are comprised of neurons and astrocytes, and primary monocytes are added to the organoid to allow for productive HIV-1 infection. The monocytes are infected with HIV-1 ADA and viral titers are measured by ELISA for the Gag p24 protein. TNF-α is administered in varying concentrations as a latency reserving agent after ART is discontinued.

Results: We have shown that our cerebral organoid models can be productively infected with HIV-1, as p24 concentrations in the media have reached up to 15 ng/mL. Monocytes cocultured with cerebral organoids produce higher viral titers than monocytes cultured alone, displaying the importance of understanding the brain microenvironment in HIV-1 replication. Additions of the antiretrovirals Raltegravir and Nelfinavir at 1uM each suppress viral replication in our model. In one experimental cycle for over 200 days, the untreated organoid continued to produce HIV-1 while an organoid previously treated with ARVs produced only minimal titers, indicating the establishment of latency.

Conclusions: Although peripheral blood analysis and animal models are highly utilized and necessary, cerebral organoids may provide a more physiological model to bridge classic in vitro experiments to human HIV-1 Cure clinical trials. Immediate future directions include optimizing monocyte penetration into organoids, evaluating additional latency reactivation strategies and adding HIV-1 specific CD8+ T cells to target these

reservoirs. HIV-1-induced neuronal and astrocyte cytotoxicity is also being measured.

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PP 2.8 – 00124

Spironolactone Represses HIV-1 Driven Transcription in Human Microglia and T cell Models of Latency and Alters DNA Methylation of Metabolic Genes

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Background: The aldosterone antagonist, spironolactone (SP), FDA-approved for clinical use, has previously been shown to inhibit HIV infection and reactivation through Tat-dependent transcriptional mechanisms in T cells. Here, we sought to compare and contrast the effects of spironolactone on microglia and T cell HIV latency and evaluate SP-related changes upon the host epigenome.

Methods: J-Lat 5A8 T cell and HC69.5 hμglia/HIV clones were treated with SP and stimulated with either PMA/ionomycin or TNF-α respectively. HIV-1 reactivation was measured kinetically via GFP reporter expression on the CytoSMART Lux3 FL and the BioTek Cytation 5 live-cell imagers to quantify fluorescence of the GFP reporter over a period of 48 hours. Genome-wide DNA methylation was assessed in J-Lat 5A8 spironolactone treated and untreated cells using the Illumina Infinium MethylationEPIC Array.

Results: Continuous SP treatment significantly inhibited HIV proviral reactivation in both J-Lat 5A8 and HC69.5 cell lines in a dose dependent manner. Pretreatment and washout of spironolactone delayed J-Lat 5A8 reactivation but was not sufficient to inhibit HIV reactivation suggesting continuous exposure to spironolactone is required to suppress transcription. DNA methylation analysis of J-Lat 5A8 treated with spironolactone revealed alterations in CpG methylation of metabolic genes compared to untreated controls. We observed differentially methylated CpG sites in genes related to the mTOR signaling pathway complimentary to previous reports of the modulatory effects of spironolactone on the mineralocorticoid receptor (MR).

Conclusions: SP treatment alone is sufficient to inhibit HIV proviral reactivation in both T cell and microglia latency cell models with continuous exposure required to suppress transcription. Alterations to CpG methylation in metabolic genes suggests a potential role for host metabolism in promoting HIV latency driven by SP. Further studies of long-term spironolactone as a component of a block-lock strategy in PLWH are warranted.

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PP 2.9 – 00125

Impact of latency reversal agents on estrogen receptor alpha gene and protein expression

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Background: Modulation of HIV and type I interferon gene transcription by estrogen receptor alpha (ERα) is reported to contribute to sex differences observed in HIV infection, clinical progression, and reservoir size. However, the impact of latency reversal agents (LRAs) being explored as part of an HIV cure strategy on ERα is unknown.

Methods: To obtain a better understanding of the influence of LRAs on ERα regulation and potentially ERα-dependent gene transcription, we evaluated the impact of different LRA classes (HDAC and BET inhibitors, PKC and STING agonist, SMAC mimetic) on ERα at both the mRNA and protein levels by qPCR and WB on PBMC and CD4+ T cells from female and male donors. All experiments were performed in hormone-free media.

Results: Sex-differences in ERα expression at both the basal level and after LRAs treatment was not observed. In both PBMC and CD4+ T cells, we observed a significant downregulation of ERα gene expression following 6-hour and/or 24-hour of treatment with all tested LRAs. In CD4+ T cells, profound ERα mRNA downregulations were observed with romidepsin after 24-hour treatment (0.02-fold change; $P < 0.01$), with PMAi after both 6-hour and 24-hour treatment (0.04-fold change; $P < 0.01$) and with PEP005 after 6-hour treatment (0.21-fold change; $P < 0.001$). AZD-5582 downregulated ERα mRNA expression the least (0.73-fold change; $P < 0.001$). Downregulation of ERα gene expression by LRAs resulted in temporal decreased of ERα protein after 6-hour treatment with PMAi (0.57-fold change; $P < 0.05$) and after 24-hour treatment with PMAi, PEP005 and romidepsin (0.37-, 0.57- and 0.77-fold change; $P < 0.001$, $P < 0.01$ and $P < 0.001$, respectively).

Conclusions: We show for the first time that current LRAs used in HIV cure studies have an impact on both ERα gene expression and protein levels. These results suggest a common influence of LRAs on the ERα pathway, which could in turn alter ERα-dependent gene transcription and merits further investigations and consideration in HIV cure studies.

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PP 2.11 – 00144

Transcriptional and translational SIV profiles of peripheral and lymphoid CD4+ T cells of viremic and ART-suppressed Rhesus macaques

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Background: Studies of viral reservoir in tissues are limited and challenging in humans. SIV-infected Rhesus macaques progress to AIDS by mechanisms similar to those observed in humans infected by HIV-1, which makes them important models for studying the pathophysiology in blood and lymphoid tissues, both in the presence or absence of antiretroviral therapy (ART).

Methods: We developed a single-cell flow cytometry fluorescent in situ RNA hybridization (RNAflow FISH) assay using multiplexed probes targeting exon, gag and nef regions of the SIV genome and p27 protein. This approach allowed detailed profiling of infected CD4+ T cells in blood and tissues (spleen, and axillary (AxiLN), inguinal and mesenteric lymph nodes (MLN) of SIVmac239-infected chronic progressors (n = 11), ART suppressed (n = 3) and uninfected (n = 6) Rhesus macaques. We used a 22-parameter flow cytometry panel on a BD FACS Symphony.

Results: In chronic progressors, frequencies of viral RNA+ T cells (vRNA+) were similar across all compartments (p = 0.342). 50% of these transcriptionally competent cells did not express p27 (vRNA+p27- T cells) whereas the other 50% were also transitionally competent (vRNA+p27+ T cells). Infected cell frequencies were strongly positively correlated between tissues (i.e MLN vs. AxiLN, r = 0.821 and p = 0.034) but there was a weaker association with blood (i.e MLN vs. blood, r = 0.619 and p = 0.115). Only frequencies of vRNA+ T cells from blood and AxiLN were associated with viral loads (r = 0.765 and p = 0.002; r = 0.929 and p = 0.007 respectively). vRNA+ T cells were enriched in central memory T cells from the blood (p = 0.024) and the spleen (p < 0.001). In ART-suppressed animals, we found higher proportions of cells with ongoing viral transcription (leaky latency cells) in tissues (3/3 spleen and AxiLN samples, 1/1 MLN samples) than blood (1/3 samples). 100% of leaky latency cells on ART carried short/abortive transcripts and did not translate p27 protein.

Conclusions: These results highlight the potential of this single-cell cytometric approach to profile reservoir compartmentalization and phenotype, demonstrating virological and immunological differences between blood and tissues that could impact reservoir establishment. On ART, we detected leaky latency cells mainly in tissues. These detailed analyses can improve understanding of the reservoir cell landscape across tissues, a critical step for cure strategies in humans.

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PP 2.12 – 00153

eCD4-Ig-DNA Decreased HIV Reservoir and Delayed Viral Rebound Through Fc Mediated Functions in BLT Humanized Mice

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Background: AAV-delivered eCD4-Ig can prevent HIV infection with more breath than broadly neutralizing antibodies (bNAbs). However, the effects of eCD4-Ig on HIV persistence during antiretroviral therapy (ART) and post-ART cessation have not been studied. In this study, we tested DNA Launched-eCD4-Ig (DL-eCD4-Ig) co-delivered with IgE-TPST2 in HIV-infected bone marrow-liver-thymus (BLT) humanized (hu) mice under ART suppression and during ART interruption.

Methods: Four independent batches of BLT hu-mice (including IL-15 transgenic hu-mice) were infected with HIVsuma (transmitted/founder virus). Following 4 to 6 weeks of viremia and suppression on cART (FTC+TDF+RAL), DL-eCD4-Ig (intact or Fc region LALA mutated) was delivered intramuscularly three times on and off ART. In-vivo expression of DL-eCD4-Ig was detected by ELISA. Plasma viral load, leukocyte subsets and activation were measured weekly. Proviral HIV DNA load (in PBMCs) during ART was measured by qPCR. Differences between and within groups were determined by two-sided nonparametric

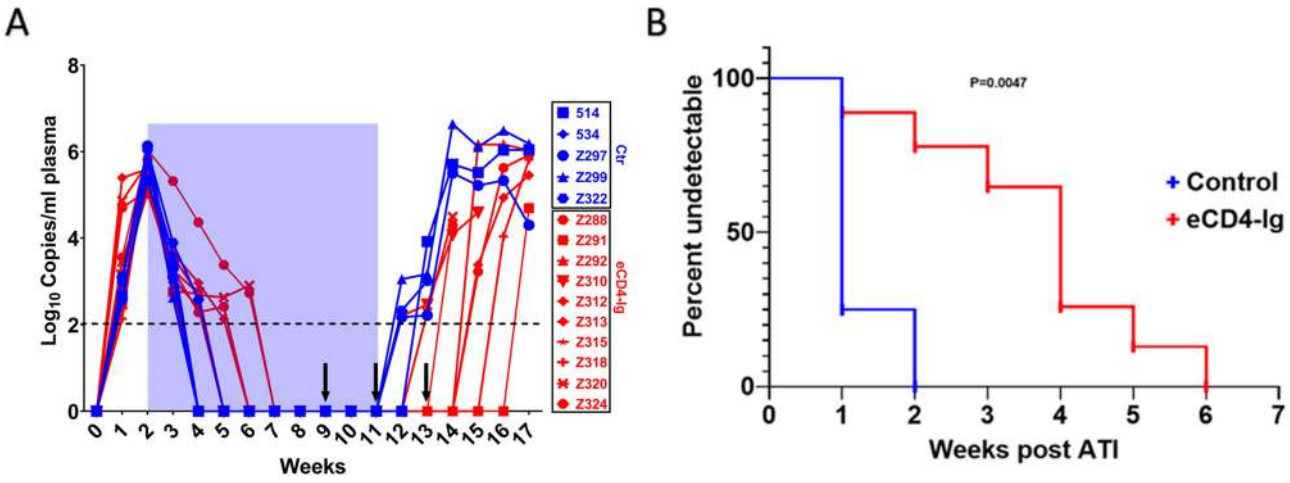

Figure 1 (abstract: PP 2.12–00153) (A) Viral rebound after ATI and (B) survival curve

tests. Survival analysis was used to evaluate the time of viral rebound between groups.

Results: Plasma from hu-mice with DL-eCD4-Ig elicited strong antibody-dependent NK cellular cytotoxicity (ADCC) against HIV-infected cells. During ART, DL-eCD4-Ig treatments elicited a significant decrease in levels of cell-associated HIV DNA ($p = 0.0355$), compared to control hu-mice. After ART interruption, DL-eCD4-Ig resulted in a significant delay ($p = 0.0047$) of time-to-viral-rebound, compared to control hu-mice. Using L-15 transgenic hu-mice with greater NK cell reconstitution, we confirmed the DL-eCD4-Ig-mediated delay of viral rebound ($p = 0.0004$); however, LALA mutation version of eCD4-Ig abrogated the effect on rebound delay ($p = 0.082$). These results indicate that the anti-viral effects of DL-eCD4-Ig are mediated significantly through an Fc-mediated immune effector mechanism.

Conclusions: DL-eCD4-Ig co-delivered with IgE-TPST2 reduced levels of cell-associated proviral HIV during ART and delayed viral rebound after ART interruption in vivo. These effects of DL-eCD4-Ig on HIV persistence and rebound are mediated through an Fc-mediated mechanism. Together, our results support that DL biologics including-eCD4-Ig may be an important strategy to reduce HIV burden on ART as a strategy for or ART-free remission and or eradication.

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PP 2.13 – 00160

Enhancing Tolerability and Efficacy of Latency Reversing Agents in "Kick and Kill" HIV Cure Approaches

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Background: The latent HIV reservoir consists of long-lived cells harboring non-expressing but functional integrated proviruses, which represent a key hurdle to HIV eradication efforts. One approach for eliminating these cells is with latency reversing agents (LRAs), which induce expression of viral proteins, allowing the host cells to be recognized and removed by viral cytopathic effects or the immune system. PKC modulators including Bryostatin-1 and synthetic bryostatin analogs are promising lead LRAs but can also cause cytokine upregulation in peripheral blood mononuclear cells (PBMCs). Importantly, some of the induced cytokines have been shown to independently cause latency reversal. The goal of the current study was to evaluate the indirect effects of PKC modulators on latency reversal, and test new synthetic compounds for their HIV latency reversal capacity.

Methods: To determine whether cytokines or other secreted factors induced by PKC modulators may contribute to latency reversal, primary human PBMCs (6–8 donors) were treated with bryostatin-1 or the designed, synthetic bryostatin analog SUW133 for 24 hours *in vitro* and after removing the compound, the replaced supernatant was collected 24 hours later. This supernatant was then analyzed for the presence and concentration of 38 different human cytokines and chemokines and was evaluated for its ability to induce latency reversal in J-Lat cell lines. To determine whether newly synthesized pro-drugs, analogs, and new scaffolds of PKC modulators can induce HIV latency reversal, these compounds were evaluated *in vitro* using latently-infected cell lines.

Results: Several cytokines were up-regulated in LRA-treated conditioned media. However, exposure of latently-infected cells to this supernatant did not result in latency reactivation. Several newly evaluated synthetic PKC modulators efficiently reversed latency in cell line models, including slow-release prodrug formulations.

Conclusions: These results indicate that production of secreted factors induced by bryostatin-1 and SUW133 in PBMC does not directly contribute to HIV latency reversal *in vitro*. The generation of both newly synthesized pro-drug compounds and analogs of PKC modulators may lead to more efficacious and better tolerated latency reversing agents with the potential to contribute to HIV cure efforts.

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PP 2.14 – 00162

Elucidating the effects of combination therapy with Venetoclax and IAP inhibitor AZD5582 in SIV-infected, ART-suppressed macaques

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Background: Combining the IAP inhibitor AZD5582 with the Bcl-2 inhibitor Venetoclax (ABT199) to reverse latency and enhance clearance of infected cells via apoptosis is a novel approach to cure HIV. In this pilot study, we evaluated the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of ABT199 with AZD5582 in a nonhuman primate model.

Methods: Two juvenile rhesus macaques (RMs) were orally infected with SIVmac251 and ART was initiated 4 wpi. At 112 wpi, RMs received escalating single doses of ABT199 at 2.5 mg/kg, 10 mg/kg and 20 mg/kg intramuscularly (i.m.). Next, AZD5582 was administered once intravenously at 0.1 mg/kg with 4 daily doses of ABT199 (at 15 mg/kg i.m.). PK parameters in plasma was evaluated and changes in absolute T- and B-cell counts were assessed by flow cytometry.

Results: Venetoclax was well tolerated, with only mild adverse events (AEs) at 10 and 20 mg/kg single doses and no AEs with repeated doses given in combination with AZD5582. AEs included short term injection site pain and swelling, with nausea and vomiting in 1 RM (20 mg/kg dose). Steady state C_{max} after 15 mg/kg i.m. ABT199 dose 4 (2.26 μg/ml) approximates the C_{max} in humans after 400 mg oral dosing (2.1 ± 1.1 μg/ml), with similar AUC_{0-24} but shorter $T_{1/2}$ observed. Absolute

CD20+ B-cell counts were used as a PD marker, with reduction of 62–91% found at 24 h post ABT199 dose 1 (15 mg/kg) + AZD5582 and full recovery to baseline levels by 2 weeks post ABT199 dose 4. Absolute CD4+ and CD8+ T-cell counts also declined at 24 h post ABT199 dose 1 + AZD5582, including both naïve and memory subsets, without complete recovery by 2 weeks post ABT199 dose 4.

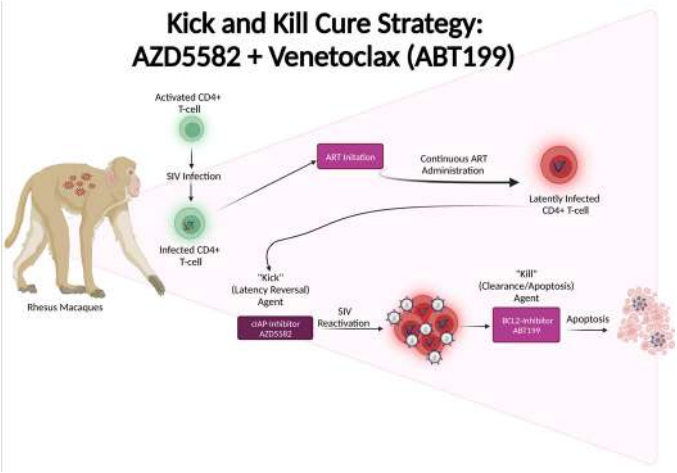

Figure. Kick and Kill Cure Stratergy: AZD5582 + Venetoclax

Conclusions: Venetoclax can be safely administered to RMs alone and in combination with the AZD5582. An immunologic response was achieved, including depletion of memory and naïve CD4+ T-cells, supporting the use of Venetoclax as a strategy to clear viral reservoirs. An efficacy study to evaluate latency reversal and apoptosis of infected CD4+ T-cells using combination therapy with AZD5582 and Venetoclax in ART-suppressed RMs is underway.

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PP 2.15 – 00169

Macrophages are the primary source of virus in semen and male genital tract organs in acutely and chronically infected rhesus macaques

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Background: Most new HIV infections result from sexual interactions with infected but untreated individuals. Semen is the main vector for viral transmission globally, however, little is known regarding the anatomic origin and form of virus in semen.

Methods: In this study, we were able to combine numerous new technologies to characterize the virus present in the semen during SIV infection. Six rhesus macaques (RM) were challenged intravenously with barcoded virus SIVmac239M. Semen and blood samples were collected longitudinally for 17 days post-infection with all male genital tract (MGT) and multiple lymphoid tissues collected at necropsy and subjected to quantitative PCR, next generation sequencing of the viral barcode, and tissue analysis (RNAscope, DNAscope and immunophenotyping). Semen was also collected from 6 animals chronically infected with SIVmac251 and in five CD4 depleted animals in acute phase and 2 weeks post ART initiation.

Results: Extremely high levels of viral RNA (vRNA) were detected in seminal plasma (up to 10^9cp/ml) as well as comparable levels of cell associated vRNA and vDNA in seminal cells with detection starting as early as 4 days post-infection. RNAscope and immunophenotyping of seminal cells and MGT tissues revealed myeloid cells as the main source of virus (Fig. 1), while CD4+T cells were harboring vRNA in lymphoid tissues. Sequences show evidence of an early compartment between seminal and blood plasma and no difference in the env gene of virus present in semen/MGT and in Lymph Nodes. Finally, multinuclear giant cells harboring vRNA were the only source of virus in semen in chronically infected and in CD4 depleted RM. Moreover, vRNA + myeloid cells were highly present in semen after 2 weeks on ART.

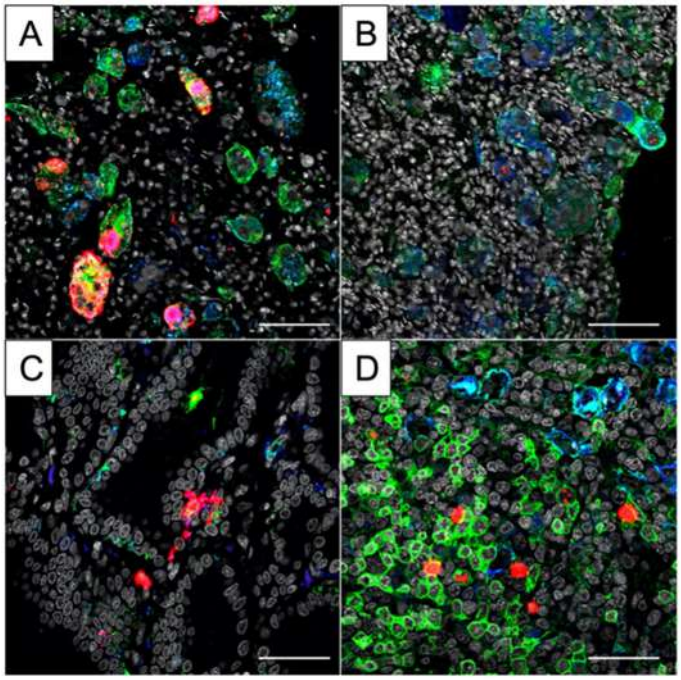

Figure. vRNA and vDNA + Myeloid cells in semen and MGT.

Conclusions: This study demonstrates the feasibility of tracking the anatomic origins of seminal virus in SIV infection and could provide novel characterization of the virus that drives sexual transmission globally. Extraordinarily high seminal VLs and massive numbers of infected cells might be associated with primary infection persisting after 2 weeks of ART and if this occurs in humans, could help explain the high rate of transmissibility during primary infection and highlights the risk of viral transmission during analytical treatment interruptions.

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PP 2.16 – 00177

Development of a nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women

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Background: The burden of human immunodeficiency virus (HIV) infection is disproportionally shouldered by transgender women (TGW) who have a global HIV infection prevalence of 19% and an estimated 66-fold higher incidence of acquisition than non-transgender individuals over age 15. Considering TGW's high risk of acquiring HIV, they stand to benefit greatly from anti-HIV therapeutics, though little work has been devoted to understanding the immunomodulatory effect of feminizing hormone therapy (FHT). To relieve gender dysphoria and take steps toward gender affirmation, many TGW utilize FHT, which consists of exogenous estrogens, primarily 17-β estradiol (E2), to facilitate physical feminization and bring estrogen levels to those of cisgender women. It is well established that estrogen can influence T-cell development and impart higher resistance to infection in women versus men. Since estrogen can also amplify antibody responses, FHT could have both positive and negative immune consequences to TGW. FHT may, for example, increase the pool of HIV target cells on one hand, but enhance the immunogenicity of HIV vaccines on the other. FHT may also influence the size of the latent reservoir in HIV-infected persons, since estrogen is a known transcriptional inhibitor of HIV. Hence, to advance our understanding of the immunomodulatory effects of FHT in TGWs, we set out to model FHT in rhesus macaques (RMs). Not only are RMs genetically similar to humans, but they make it possible to conduct longitudinal studies with ensured treatment compliance and access to precious mucosal samples.

Methods: Using slow-release subcutaneous E2 pellets, we have optimized a dosing regimen in male RMs that maintains similar levels of E2 to those measured in cisgender women. We are using flow cytometry to measure cellular dynamics and intracellular cytokine staining assays to measure cellular responses to HIV vaccines.

Results: Not only does this E2 pellet treatment induce physical feminization (i.e., increased nipple sizes), but it also triggered fluctuations in activated CD4+ T-cells and NK cells over time.

Conclusions: Collectively, this work highlights the pleiotropic effects of E2 and provides a new translationally relevant *in vivo* system to explore the immune consequences of exogenous hormone therapy in TGW.

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PP 2.17 – 00187

Myeloid-derived extracellular vesicle production is upregulated with SHIV.D infection

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Background: While the successes of combination antiretroviral therapy (cART) have minimized the number of people with human immunodeficiency virus (HIV) (PWH) that progress to acquired immunodeficiency syndrome (AIDS), PWH on cART maintain at an increased risk for HIV-associated neurological dysfunction. Extracellular vesicles (EVs) constitute an understudied method of intercellular communication and molecule delivery in viral infections. EVs have been demonstrated to carry inflammatory mediators to areas of the periphery during cART suppression but are understudied in the brain. Here, we utilize novel myeloid-tropic transmitted/founder simian-human immunodeficiency virus SHIV.D to investigate myeloid-derived EVs.

Methods: Primary rhesus macaque (RM) monocyte-derived macrophages (MDMs) were infected with 13.5 ng SHIV.D p27/mL. Cell culture supernatant was collected after 72hrs infection. Free virions were removed by immunoprecipitation. EVs were isolated and analyzed by ZetaView nanoparticle tracking. Cellular DNA was isolated for SHIV.D pol and RM telomerase reverse transcriptase (TERT) qPCR.

Results: EV concentration normalized to RM TERT was significantly increased in SHIV.D infected MDMs compared to uninfected MDMs from the same RM donor ($P = 0.03$, $N = 6$). There was no significant difference in the size of the EVs from SHIV.D-infected MDMs compared to controls. Preliminary flow cytometry analysis of EVs from SHIV.D-infected rhesus macaque plasma showed an overrepresentation of monocyte-derived EVs compared to T-lymphocyte-derived EVs.

Conclusions: Ongoing research will compare EV production, cellular origin, and EV cargo from SHIV.D-infected RM plasma, cerebral spinal fluid, and tissues prior to infection, and during acute viremia and cART suppression. Discovering the role of myeloid-derived EVs on the continuous neuroinflammation during suppressive cART in the SHIV.D/RM model is a critical step in developing novel interventions to combat HIV CNS dysfunction.

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Session 3: Virology of HIV Persistence

OP 3.1
HIV Persistence in women, an update

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The persistence of HIV-1 infection despite effective antiretroviral therapy (ART) still requires lifelong ART, but efforts are moving steadily forward towards viral eradication or durable drug-free remission. While women constitute one half of people living with HIV disease, remarkably a systematic analysis demonstrated that only 11.1% of cure study participants in the past 17 years have been female. Sex-based differences may influence the outcome of HIV infection and similarly the HIV reservoir. During early infection, untreated HIV+ women exhibit levels of plasma viremia up to 40% lower than men and higher CD4 T cell counts. However, women are at 1.6-fold higher risk to progress to AIDS than men. Factors specific to women, such as differences in innate and adaptive immunity, genetics and cyclical hormonal changes, may affect HIV pathogenesis, immune function, and the reservoir. Reservoir size, latency reactivation and immune clearance are each critical factors in one of the proposed paths to HIV remission. Each of these may differ between men and women. Recent studies exploring HIV persistence and cure in women along with associated sex differences will be highlighted.

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OP 3.2 – 00056
Clonally expanded HIV-1 proviruses with 5′-Leader defects can give rise to nonsuppressible residual viremia and complicate ART management

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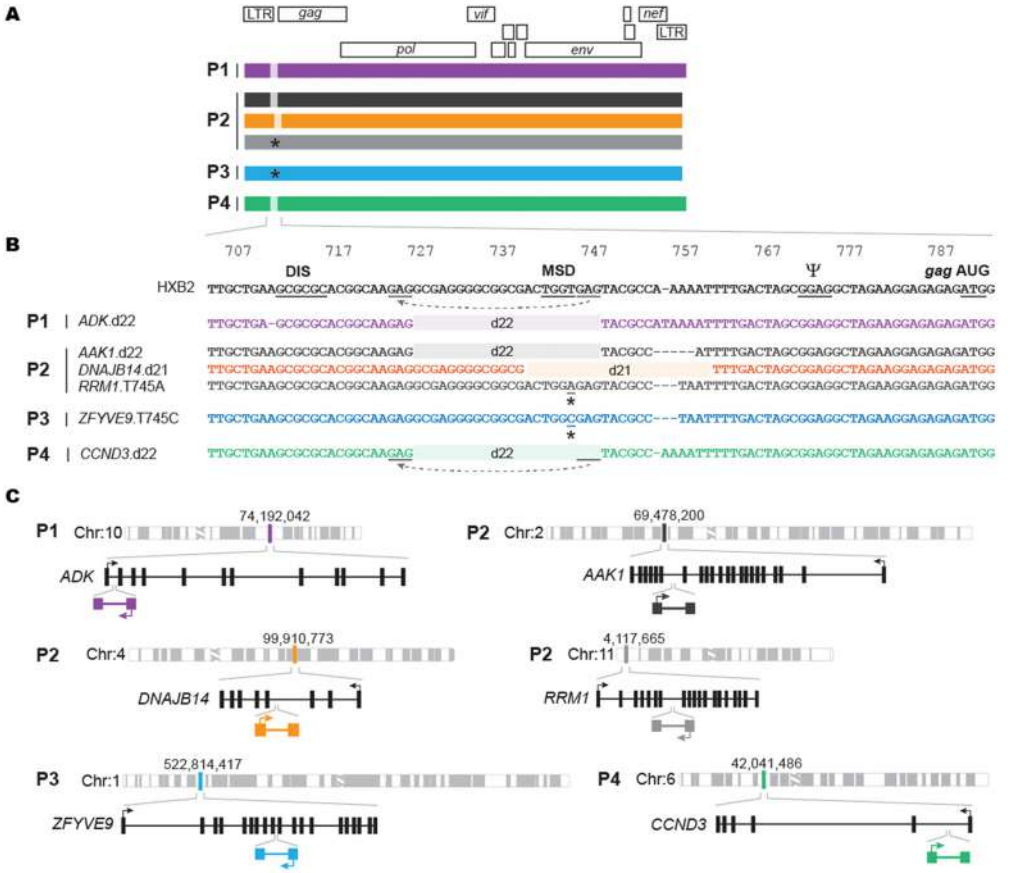
Background: Antiretroviral therapy (ART) decreases viremia to below the detection limit of clinical assays in people living with HIV. Most proviruses are latent, but, at any given time, a small



Paired full genome sequencing and integration site analysis of 5'-Leader-defective proviruses causing NSV. (**A**) Mapped sequences of proviruses contributing to plasma HIV-1 RNA; light-shaded areas indicate small 5'L deletions; star symbols indicate point mutations. (**B**) 5' Leader defects aligned to the HXB2 reference; key RNA features are underlined and labeled in bold: DIS dimerization initiation signal, MSD major splicing donor, PSI packaging signal, AUG *gag* start codon; grey lines highlight GAG repeats causing misplaced jumping during RT (dashed grey arrows). (**C**) Chromosomal and gene locations of proviruses causing viremia.

Figure 1 (abstract: OP 3.2–00056) Proviruses cause of NSV have 5′-Leader defects

fraction expresses RNA and virions. In some individuals this causes persistent nonsuppressible viremia (NSV) originating from clones carrying infectious proviruses. Defective proviruses represent over 90% of proviruses persisting during ART and can express viral genes. Whether defective proviruses can cause NSV and complicate ART management is unknown.

Methods: We characterized the source of NSV in 4 study participants with 100% adherence to ART. We performed SGS on plasma RNA and CD4 gDNA, IPDA, and matched integration site and provirus sequencing. We studied inducibility and splicing ex vivo. 5′-Leader defects were introduced in NL4-3 to test RNA dimerization, nucleocapsid binding, infectivity, Env expression, and RNA splicing. Integration site-specific assays were used to track clones of interest over time and in cell subsets.

Results: We identified 6 proviruses with 5′-Leader defects, part of expanded clones, contributing to persistent NSV ranging from ~10^2 to ~10^3 HIV RNA copies/mL. These proviruses have small deletions or point mutations involving the major splicing donor site (MSD). We found an identical deletion (d22) in 3 out of 4 participants, likely favored by GAG repeats surrounding the MSD. 5′-L defects partially reduced RNA dimerization and nucleocapsid binding. Nevertheless, proviruses are inducible and produce non-infectious virions containing viral RNA but lacking Envelope due to inefficient alternative splicing. Proviruses cause of NSV were all integrated into and in the opposite orientation of expressed genes, showed variable degrees of CTL escape mutations, and were resistant to autologous neutralization. These infected clones have variable sizes, are stable over time, and are siloed in effector memory cells. In one participant, a >50-fold expansion of one clone occurred concurrently with the onset of NSV.

Conclusions: Despite defects affecting critical processes including the generation of spliced transcripts, proviruses with small 5′-Leader defects in expanded CD4+ T cell clones can give rise to NSV, affecting clinical care.

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OP 3.3 – 00030

Inducible replication-competent HIV proviruses persist in memory CD4+ T cells expressing high levels of the integrin VLA-4 (α4β1)

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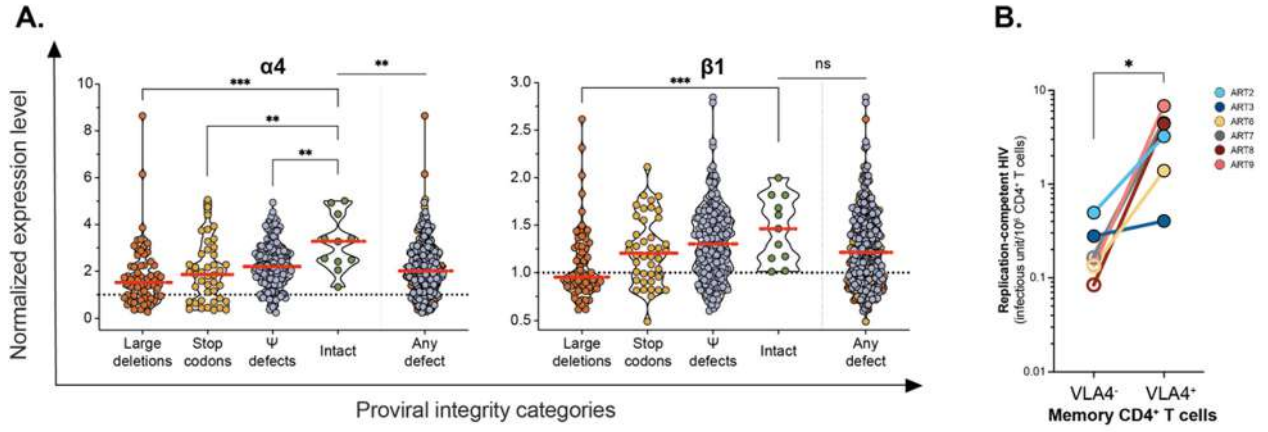
Background: Even after years of suppressive antiretroviral therapies (ART), viral reservoirs persist in people living with HIV (PLWH). We aimed at identifying cellular markers preferentially expressed by the few cells in which inducible, intact, and replication-competent proviruses persist during long-term ART.

Methods: We used HIV-Flow indexed p24+ single-cell sorting and combined it with near full-length (NFL) HIV sequencing to obtain the phenotype of cells harboring inducible and intact proviruses in 6 participants on ART. Specifically, we measured the expression levels of PD-1, TIGIT, HLA-DR, ICOS, α4, β1, CD45RA and CCR7. Markers enriching in cells with intact proviruses were validated by cell-sorting of memory CD4+ T cells from 6 PLWH on ART. Measures of replication-competent HIV were performed on the sorted populations by viral outgrowth assay.

Results: Among 309 NFL proviruses sequenced from p24+ cells, only 12 were genetically intact. Intact HIV genomes were integrated in memory CD4+ T cells expressing higher levels of the integrin subunits α4 and β1 (i.e. integrin VLA-4), when compared to cells harboring defective proviruses (normalized expression level of α4 of 3.3 on intact VS 2.0 on any defects; p = 0.003, and 1.5 VS 1.2 for β1; p = 0.06) (Fig.1 A). No difference was noted for all other markers. Viral outgrowth assay confirmed that memory CD4+ T cells expressing high levels of VLA-4 were dramatically enriched (27-fold) in replication-competent virus (mean: 3.24 VS 0.12 IUPM for VLA4-; p = 0.031) (Fig.1 B).



A. Normalized expression level (Fluorescence intensity of the maker on each single-sorted p24+ cells/MFI of CD4+ population) of α4 and β1 on infected cells, grouped by their proviral integrity. **B.** Replication competency of memory CD4+ T cells expressing high levels of α4β1 (VLA4+) or not (VLA4-) from 6 ART-treated PLWH.

Figure 1 (abstract: OP 3.3–00030)

Conclusions: Our results indicate that the inducible, intact, and replication-competent reservoir persists in memory CD4 + T cells that express high levels of the integrin VLA-4. Whether this molecule can be pharmacologically targeted to reduce the size of the HIV reservoir remains to be determined.

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OP 3.4 – 00197

Infected naïve CD4 + T cells in children with HIV can proliferate and persist on ART

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Background: We previously showed that HIV persists in perinatally infected children through clonal expansion of Tcells infected before ART initiation. Although HIV primarily infects memory CD4 + Tcells (TMem), studies in adults and SIV/SHIV-infected rhesus macaques have shown that naïve CD4 + T cells (TNaive) harbor a higher proportion of intact HIV genomes. Little is known about the TNaive HIV reservoir in early-treated children. The study aimed to determine (i) if HIV infects TNaive in early-treated children, (ii) the infection frequency relative to TMem, (iii) the proportion predicted intact (iv) if infected TNaive can undergo cellular proliferation to form infected Tcell clones.

Methods: The cohort consisted of 8 children aged 5–11years who initiated ART at a median of 4weeks of age with suppressed viremia for a median of 8.5 years. PBMC were sorted into TNaive (CD45RO-CD28 + CD27 + CD95-CCR7 + CD45RA +) and TMem (CD45RO + CD95 +). Multiple displacement amplification (MDA) was performed on genomic DNA from 320,000 TNaive and 106,667 TMem from each child at limiting dilution for HIV proviruses. To detect MDA reactions containing an HIV provirus and estimate the proportion of the HIV + MDA reactions with predicted intact proviruses, probe-based PCR methods were used to screen for HIV LTR, Psi, and RRE. Integration site analysis (ISA) was performed on TNaive in the child with the highest TNaive frequency of infection.

Results: FACS sorting resulted in purities of a median 96.6% (range 93–100%) TMem and 97% (range 96.5–100%) TNaive. HIV-infected TNaive were detected in all 8 children at a median of 37.5 infected cells/million (range 6–231), a mean of 11-fold lower than the infected TMem. of the 201 HIV LTR + TNaive detected, 4 were predicted intact (6.5% of proviruses with detectable Psi and/or RRE, 2% of LTR +). ISA identified 8 clones of infected cells in the TNaive. None of 8 infected cell clones were found to carry intact HIV proviruses.

Conclusions: We found infected TNaive persisting in children with perinatal HIV on ART for 5–11years. Infected TNaive can proliferate into clones of infected cells. Measurements adapted from the Intact Provirus Detection Assay, showed that 6.5% of infected TNaive (2% of LTR +) are predicted intact. Our results demonstrate that TNaive are an important HIV reservoir in perinatally infected children on ART.

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OP 3.5 – 00152

HIV-1 RNA + infected CD4 T cell burden in acute HIV-1 infection and association with inflammatory markers

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Background: Early events during HIV-1 infection are thought to influence subsequent virologic and immunologic outcomes, such as reservoir size, adaptive immune responses, and persistent immune activation. Robust CD4 T cell infection and inflammatory responses are hallmarks of acute HIV-1 infection, yet detailed characterization of these early events remains poor. We aimed to determine the extent of CD4 T cell infection by cell-associated viral RNA (vRNA), assess the phenotype of vRNA + infected cells and relate these findings to inflammatory cytokine production at the earliest stages of HIV-1.

Methods: HIV-1 vRNA + memory CD4 T cells in PBMC (n = 24) and lymph nodes (n = 4) of people with Fiebig stages II–V acute HIV-1 in Bangkok, Thailand (RV254) were measured by surface staining, flow cytometric limiting dilution sorting, and sensitive RT-qPCR for spliced (env/vpu/nef, tat/rev) unspliced (gag), and transcriptionally complete (LTR.U3) vRNA. Plasma levels of 27 soluble inflammation markers were assessed by Luminex or ELISA. Single-cell profiling of vRNA + cells was performed for two participants.

Results: A median of 4% of memory CD4 T cells contained unspliced vRNA (range 0.1–30%), indicating widespread CD4 T cell infection. Replication-active cells, i.e. spliced vRNA + , were ~0.2% of memory CD4 T cells (range < 0.01–1.6%), or 5% of all vRNA + cells. The proportion of vRNA + cells with 5′ unspliced vRNA was similar to that with distal transcribed vRNA, suggesting robust transcriptional elongation during acute infection and/or frequent cellular incorporation of virion-derived genomic vRNA. Unspliced vRNA + CD4 T cells correlated with plasma viremia and elevated concentrations of IL-12p70 (rho = 0.7, PFDR = 0.01), a key regulator of cell-mediated immunity. A strong trend was also observed for Mip3b. vRNA + cells exhibited a heterogeneous surface protein profile, including both resting and activated cells with varied chemokine receptor expression.

Acknowledgements: We would like to thank the study participants who committed so much of their time for this study. The participants were from the RV254/SEARCH 010, which is supported by cooperative agreements (WW81XWH-18-2-0040) between the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., and the USA Army Medical Research and Development Command (USAMRDC) and by an intramural grant from the Thai Red Cross AIDS Research Centre and, in part, by the Division of AIDS, the National Institute of Allergy and Infectious Diseases, National Institutes of Health (AAI21058-001-01000). Antiretroviral therapy for RV254/

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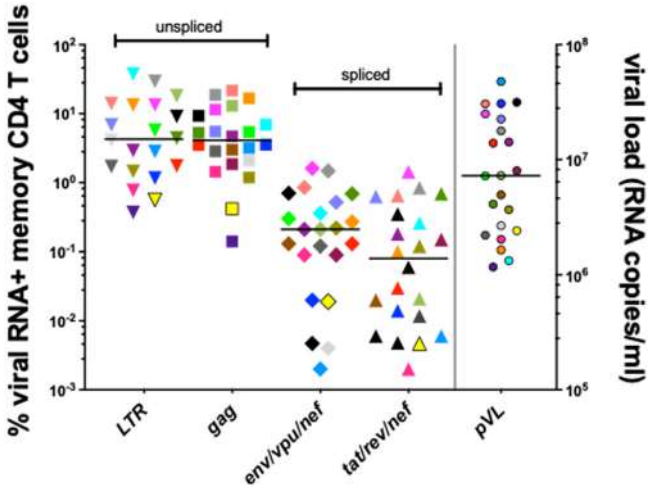

Figure. Frequency of HIV-1 RNA+ memory CD4 T cells

Conclusions: These data suggest that a substantial fraction of CD4 T cells is infected during early acute HIV-1 but that most cellular infection events lack evidence of viral replication. The diverse phenotype of early infected cells poses challenges for targeted therapeutic approaches aiming to eliminate them. CD4 T cells harboring vRNA may be an important determinant of the pro-inflammatory state regulating innate and adaptive immune response development.

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OP 3.6 – 00023

Effect of HIV-1 infection, viral particle production, and proviral integration site on CD4+ T cell proliferation

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Background: The latent reservoir (LR) in resting CD4+ T cells is the major barrier to cure. In HIV-infected individuals on long-term ART, many CD4+ T cells in the LR are clonal and have arisen from proliferation. Consequently, clonal expansion is a major mechanism of HIV persistence. Stimulation of latently-infected CD4+ T cells results in the nuclear translocation of transcription factors required for both cellular activation and reactivation of the latent provirus. These two transcriptional programs can result in opposing cell fates, cell division and survival or virion production and cell death. We asked whether proviral intactness, viral particle production, and HIV-1 integration site affect the fate of an individual infected CD4+ T cell.

Methods: We validated a high-throughput assay to isolate individual latently-infected resting memory CD4+ T cells in a background of uninfected cells from peripheral blood of 10 PLWH suppressed on ART for >7 years. Cells were stimulated with anti-CD3/CD28 beads and IL-2 to mimic antigenic stimulation. ART was included in cell culture media to prevent reinfection. At the end of culture, clonal expansion and viral particle production were quantified and proviral intactness and integration site were determined.

Results: Uninfected T cells and T cells with a defective HIV provirus proliferated ten and four times better, respectively, than T cells with an intact HIV provirus. HIV-1 integration into a cancer- or proliferation-associated gene endowed a proliferative advantage. Infected cell clones that produced >100,000 viral particles had a proliferative disadvantage. However, viral particle-positive clones did not display inferior proliferation overall compared with viral particle-negative clones. Moreover, >80% of infected cell clones produced no viral particles. Unexpectedly, viral particle production was not strongly correlated with poor infected cell proliferation.

Conclusions: These findings further our understanding of overall reservoir persistence and the fate of individual latently-infected T cells upon antigenic stimulation. Clinically, these experiments suggest that anti-proliferative agents may disproportionately affect uninfected T cells. Although there is no biomarker that distinguishes infected from uninfected T cells, we observed differences in proliferation in response to T cell activation. Future studies should investigate whether dividing infected T cells transiently express viral proteins, which may be targetable.

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OP 3.7 – 00099

Cohort-specific Adaptation of the Intact Proviral DNA Assay (IPDA) to HIV-1 subtypes A1, D, and recombinants

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Background: Existing IPDA primers/probes, designed for HIV-1 subtype B, may not detect non-B subtypes, which dominate in sub-Saharan Africa. We adapted the original IPDA primers/probes to subtypes A1, D, and recombinant HIV-1 sequences from Rakai, Uganda, and evaluated their performance.

Methods: HIV-1 subtypes A1 and D proviral genomes collected from participants on suppressive ART were collected using FLIP-seq (HXB2 coordinates 632–9632) then interrogated

for the presence/absence of the published IPDA primer/probe binding regions (HXB2 psi 692–797 and env 7736–7851) and sequence diversity using an in-house bioinformatics pipeline. Based on the sequence diversity at each site, we introduced 1–5 degenerate bases into the original primers/probes to generate pan-A1/D adapted oligonucleotides. Performance was evaluated using FLIP-seq PCR amplicons representing intact and hypermutated genomes as templates.

Results: In our *in silico* analysis, 607 subtype A1, D and recombinant intact and defective HIV-1 DNA genomes were classified into psi+/- and env+/- with and without hypermutations. All (32/32) intact genomes were psi+/env+ (*i.e.,* IPDA primer/probe locations have 100% sensitivity to identify A1/D/recombinant genomes), whereas 5% (28/547) of non-hypermutated defective genomes contained both IPDA regions, yielding 95% specificity by location. All genomes however had at least one mismatch against the original primers/probes (Figure 1), and the original IPDA subtype B primer/probes failed to amplify intact A1 and D sequences. At base 13 of the IPDA env probe (which is "G" in the labeled probe, but "A" in the unlabeled competitive probe that discriminates hypermutated proviruses in subtype B), intact subtype A1 naturally harbored an "A" rather than the consensus "G" in subtype B. Our adapted A1D primers/probes, when tested on four unique pure templates, correctly amplified intact A1 and D genomes, but not A1 and D hypermutated amplicons, though it required a slanted gate to separate positive and negative droplet populations.

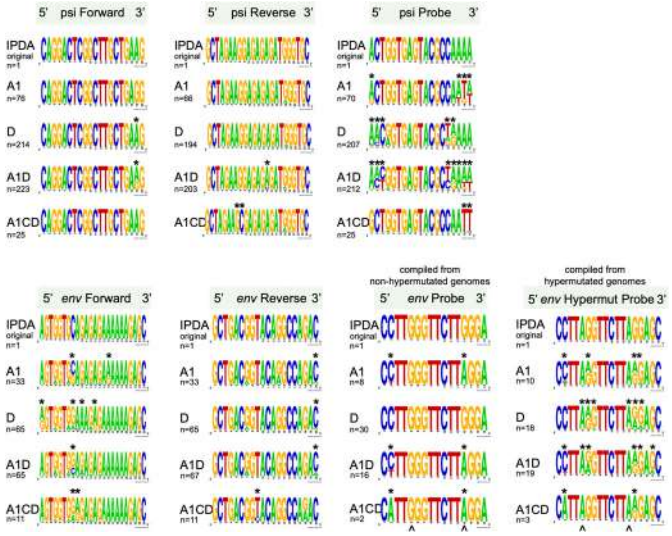

Figure. Ugandan sequence diversity at IPDA binding sites

Conclusions: Our subtype-A1/D adapted IPDA primers/probes successfully amplified genome-intact pure templates while excluding hypermutated ones. The primer/probe locations were predicted to be >95% sensitive and specific for intact genome discrimination *in silico*. Efforts to apply the primers/probes to additional templates and clinical samples, and evaluate whether placing them in alternative regions will further improve specificity, are ongoing.

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OP 3.8 – 00066

Characterization of the HIV-1 viral reservoir in subtype B early treated individuals

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Background: The clonality and intactness of proviral genomes remain poorly characterized in early treated individuals on antiretroviral therapy (ART). Moreover, the inducible viral reservoir has been understudied due to the lack of potent latency reversing agents (LRA). Here, we did an in-depth assessment of the total and inducible viral reservoir in early treated individuals.

Methods: Near full-length (NFL) proviral sequences and integration sites were analyzed in 8 individuals treated during acute infection (Fiebig II-III: n = 6; Fiebig IV: n = 1; Fiebig V: n = 1) who received ART for a median of 0.96 years (0.49–1.93 years), using matched integration site and proviral sequencing (MIP-Seq). Multiplex digital PCR was used to assess total and intact HIV proviral DNA (IPDA). Following a 24h-stimulation with a Tat mimetic (Tat#1) and PMA, the frequency of p24-expressing cells and their phenotype were assessed by HIV-Flow.

Results: The median frequency of cells harboring intact proviruses per million CD4 T cells was 90.7. Clonally expanded cells were retrieved in only 4 out of 8 participants and accounted for 5% of total integration sites (n = 270). Among proviruses integrated in genes, opposite orientation relative to the host gene was approximately twice as common as same orientation (63% vs. 34%). In total, 64 proviral genomes were obtained of which 9% of the proviruses were intact (91% defects: 2% inversions, 53% large internal deletions, 17% hypermutations, 9% PSI/MSD defects, 9% premature stopcodon/frameshift). Following PMA/Tat#1 stimulation, a range of 0.4–20 p24-expressing cells per million CD4 T cells were detected among the 8 individuals. p24-expressing cells were enriched in effector memory T cells ($p = 0.06$). Interestingly, in early treated individuals, a significantly higher fraction of p24+ cells displayed a naïve phenotype compared to chronically treated individuals ($p = 0.03$).

Conclusions: To conclude, these data indicate early ART initiation does not prevent the establishment of the intact viral reservoir. After 1 year of treatment, the contribution of clonal expansion to the persistence of the viral reservoir is minimal in early treated individuals. Here, we report a potent combination of LRAs that enables to study the inducible reservoir in early treated individuals on ART.

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PP 3.1 – 00058

Development of a digital PCR assay to profile HIV expression at single-infected-cell resolution

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Background: HIV gene expression in CD4+ T cells is often used to study reservoir activity and the impact of latency-reversing

strategies. However, bulk cell measurements fail to deconvolute changes in HIV RNA at the single-cell level. Despite the plethora of new single-cell technologies, some are limited by low cell input, allelic drop out, and require pre-amplification steps. Here, we optimized a scalable yet sensitive assay to profile HIV expression with single-infected-cell resolution.

Methods: Individual or small pools of cells were distributed in 96-well plates. RNA was isolated by magnetic beads, followed by DNAse and HIV poly-A tail-anchored cDNA synthesis. A synthetic DENV RNA spike-in was used to control for recovery. cDNA was directly quantified by measuring total poly-A, unspliced 5′ gag transcripts, and the DENV spike-in. Performance was tested on a validated HIV RNA standard, singly-sorted ACH2 cells ± PMA/I, and single J-Lat 10.6 cells with low, mid, and high GFP expression.

Results: The 95% hit rate of the assay was 17.7 copies, and the absolute LOD was 2.2 copies/well. In ACH2 cells, HIV RNA was detected in 44/45 and 40/45 replicates, and median HIV RNA copies/cell were 15.8 [IQR 7.6–176.0] and 3532 [1758–7023] for untreated and PMA/I-treated cells, respectively. In J-Lat cells, HIV RNA was detected in 27/28, 10/28 and 5/28 replicates, with median HIV RNA copies/cell being 311 [38–911], 4.3 [4.2–8.5], and 4.4 [4.3–6.5] for cells with high, mid, and low GFP expression, likely reflecting GFP turnover in cells returning to latency. We then profiled HIV expression in CD4+ T cells from a participant on ART. Cells were either stimulated with PMA/I or left untreated and seeded at limiting dilution. Our approach revealed that the increase in HIV RNA from bulk-cell measurement was due to rare high RNA-producing cells (762 copies/cell), while the majority of RNA+ cells showed only a modest increase in HIV expression (median 8.8 vs 4.4).

Conclusions: Our approach can sensibly recover intracellular viral transcripts without a pre-amplification step. Its flexible design can be applied to a wide range of low-input experiments, allowing the granular characterization of HIV expression.

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PP 3.2 – 00068

Differential transcriptional levels of HIV-1 near full-length and highly deleted proviruses

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Background: HIV-infected cells during antiretroviral therapy (ART) carry intact or defective proviruses in genomes or in transcriptional/translational activity. Transcriptional activity contributes notably to differential dynamics of proviral populations. We compared and identified viral determinants affecting transcriptional levels of intact, near full-length (NFL) defective, and total proviruses after long-term ART.

Methods: Blood cells were collected from 10 HIV-suppressed patients at pretherapy and after 4–20y on ART. We performed single genome sequencing (SGS) of proviruses >7 kb by Illumina MiSeq and characterized genetic intactness. The total proviral *gag* and cell-associated RNA (caRNA) populations were obtained by SGS of 1.3 kb *gag* region. We analyzed only sequences without stop codons on *gag*. We measured gentic diversity using average pairwise distance (APD) and net divergence (%subs/site) of caRNA from NFL and from total *gag* populations. We analyzed HLA-associated escape mutations and reported mutations on proviruses with >1.5-fold difference compared to caRNA.

Results: We obtained 1291 sequences without stop codons on *gag*, ranging from 0 to 57 NFL (both intact and defective), 5–42 total *gag*, and 3–39 caRNA sequences/timepoint. There was no significant difference in the diversity of caRNA vs NFL or total proviruses from pretherapy to long-term ART. The median APD (interquartile range-IQR) of caRNA, NFL, and total *gag* populations after long-term ART were 1.66 (0.35–1.97), 1.10 (0.33–1.46), 1.39 (0.68–1.66) while NFL and total *gag* populations at pretherapy had a median APD of 1.62% and 1.64%, respectively. CaRNA were genetically closer to total *gag* than NFL populations with the median (IQR) divergence of 0.06 (0.005–0.19) vs 0.14 (0.03–0.5) ($p = 0.02$). The genetic diversity of intact was not different from NFL defective populations. Interestingly, caRNA populations were not differently divergent from intact or NFL defective proviruses, 0.04 (0.005–0.5) vs 0.2 (0.04–0.48) ($p = 0.46$). We observed a >1.5-fold difference in the frequency of some HLA-associated escape mutations in proviruses compared to caRNA in 6/10 patients (table 1).

Table 1: Selection of HLA-associated escaped epitopes in proviruses compared to caRNA populations
Overall, the frequencies of wild-type (capital) and escaped mutations (lowercase) on HLA-associated epitopes in *gag* were not significantly different in NFL vs total *gag* vs caRNA populations after long-term ART. However, 6 patients had evidence of selection for or against some epitopes in proviral *gag* compared to caRNA.

Patient	HLA	HXB2 start on gag	Wildtype epitope	Variant epitope	Frequency of total proviral gag (n/N, %)	Frequency of intact and NFL defective gag (n/N, %)	Frequency of caRNA *gag* (n/N, %)
1	B*07:02	407	APRKKGCWK	APRKrGCWK	24/25, 96.0	18/33, 54.5	17/17, 100
2	A*02:23	77	SLYNTVATL	SLfNTVATL	18/25, 75.0	24/26, 92.3	7/13, 53.9
3	A*02:31	77	SLYNTVATL	SvYNTVATL	1/5, 20.0	1/1, 50.0	9/13, 69.2
4	A*02:74	433	FLGKIWPSYK	FLGKIWPSrK FLGKIWPScK	3/19, 15.8 5/19, 26.3	2/9, 22.2 1/9, 11.1	1/25, 4.0 13/25, 52.0
5	A*30:02	76	RSLYNTVATLY	kSLYNTVATLY	14/30, 46.7	27/34, 79.4	1/3, 33.3
6	A*02:01	77	SLYNTVATL	SLYNTiATL	9/33, 27.3	4/7, 57.1	0/34, 0

Conclusions: Transcriptional levels of intact and NFL defective proviruses are not significantly different. However, total *gag* proviruses i.e highly deleted, are more transcriptionally active than NFL proviruses and the HLA escape mechanism does not explain this difference. These results suggest the advantage of highly defective proviruses in transcription elongation.

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PP 3.3 – 00071

Longitudinal proviral landscape and reservoir dynamics in a unique case of HIV superinfection

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Background: Within-host HIV genetic diversity can pose challenges to reservoir assessment, but it can also be leveraged to understand reservoir dynamics. We longitudinally characterize reservoir composition, including HIV RNA sequences rebounding in plasma during ART interruption, in an individual who initially acquired HIV subtype B and was subsequently super-infected with a unique recombinant form (URF).

Methods: Single-genome amplification (pol and env) was used to characterize plasma HIV RNA populations replicating shortly after infection in 2010, and in 2012 prior to ART. Near-full-genome proviral sequencing and reservoir quantification (IPDA/QVOA) was performed in 2017 during suppressive ART. In 2018, the participant interrupted ART, allowing us to characterize plasma HIV sequences emerging from the reservoir. Near full-genome proviruses were resampled in 2019 after re-suppression on ART.

Results: All 25 plasma HIV sequences sampled in 2010 were subtype B. By 2012 however, only 10% of sampled plasma sequences were B; the remainder were a URF that was a mosaic of subtype A1, G, and CRF02_AG. This indicates super-infection, with the URF subsequently becoming dominant. In 2017 during suppressive ART, the IPDA estimated an overall 78 intact HIV copies/million CD4+ T cells, of which 36% were subtype B and 64% were the URF (the two strains produced different signal amplitudes, allowing strain-specific quantification). Somewhat in contrast, QVOA returned an overall reservoir size of only 0.27 IUPM CD4+ T-cells, though full-length infectious viruses representing both B and URF were recovered from supernatants. We also recovered 51 near-full-length proviruses, of which 10 were intact, including 2 subtype B and 8 identical URF proviruses. After treatment interruption in 2018, 91% and 9% of the 130 plasma HIV sequences recovered were URF and B, respectively. After re-suppressing on ART in 2019, 90% of the 40 recovered near-full-length proviruses were the URF (one intact) while 10% were subtype B (zero intact).

Conclusions: HIV sequences rebounding in plasma after ART interruption reflected reservoir lineage distribution on-ART, which in turn reflected within-host variant distribution pre-ART. Results highlight reservoir longevity and genetic stability, and illustrate how HIV sequencing performed in combination with reservoir quantification can provide additional insights into reservoir composition and dynamics.

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PP 3.4 – 00079

HIV-1 clade C reservoir characteristics in early and chronic treated infection

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Background: Persisting HIV reservoir viruses in resting CD4 T cells and other cellular subsets remains a barrier to curative efforts. Early antiretroviral treatment (ART) has been shown to enable post-treatment viral control in some cases. We hypothesized that extremely early ART initiation will affect the size, decay dynamics and landscape characteristics of the HIV-1 subtype C viral reservoirs.

Methods: We studied 35 women from the FRESH cohort in Durban, South Africa diagnosed with hyperacute HIV infection by twice weekly testing for HIV-1 RNA. Study participants were divided into 2 groups where, 11 started ART at a median of 456 (297–1203) days post onset of viraemia (DPOV), while 24 started ART at a median of 1 (1–3) DPOV. We used PBMC to measure total HIV DNA by ddPCR and sequence viral genomes by full length individual proviral sequencing (FLIP-Seq) from detection of HIV up to 1 year post treatment.

Results: ART in hyperacute infection reduced peak viraemia compared to untreated infection ($p < 0.0001$), but there was no difference in total HIV DNA measured contemporaneously ($p = 0.104$). There was a steady decline of total HIV DNA in the early treated group over 1 year that was not observed in the late treated group ($p = 0.0004$). Total HIV DNA after 1 year of treatment was lower in the early treated compared to the late treated group ($p = 0.02$). We generated 697 single viral genome sequences. There was a difference in the longitudinal proviral genetic landscape over 1 year between untreated, late treated and early treated infection, where the relative contribution of intact genomes to the total pool of HIV DNA after 1 year was higher in untreated infection (31%) compared to late treated (14%) and early treated infection (0%). Treatment initiated in both late and early infection resulted in a more rapid decay of intact ($T1/2 = 2$ months and $T1/2 = 0.75$ months) versus defective ($T1/2 = 25$ months and $T1/2 = 8.54$ months) viral genomes.

Conclusions: Extremely early ART initiation in subtype C HIV-1 was associated with a more rapid decay of intact viral genomes which could accelerate reservoir clearance when combined with other interventional strategies.

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PP 3.5 – 00110

The proviral quasispecies of HIV-1

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Background: HIV-1 replication is robust during untreated infection, but infected cells that persist during therapy do not constitutively express virus. Population modeling often assumes a cellular on-off dichotomy and that the same amount of virus is released from all cells actively expressing HIV-1. We established an experimental system to assess the spectra of HIV-1 expression patterns.

Methods: Cell-autonomous variation in HIV-1 expression was examined using pools of barcoded integrants in cultured cells analyzed by high-through sequencing of released virus and intracellular RNA expression patterns. How this variation contributes to proviral population remodeling under differing environmental conditions was tested.

Results: Integrant clones' burst sizes spanned four orders of magnitude. Bimodal expression patterns were established within each clone, with some cells producing no virions and other cells in the same clone expressing virus. Some burst size variation among clones reflected intracellular RNA abundance, but differences in splicing patterns also contributed. Reasoning that this variation constituted a quasispecies from which differing subsets of proviral clones could prevail under differing environmental conditions, proviral populations were compared in the presence and absence of the cytotoxic HIV-1 protein Vpr. The results showed that whereas most infected cell clones were dominated by HIV-1 expressing cells in the absence of vpr, its presence selected for a subset of clones in which a small minority of member cells released virus, regardless of the amount of virus active cells released. vpr-populations rendered vpr+ by complementation yielded similar phenotypes, suggesting clone-associated phenotypes were not due to somatic variation among infected cells. Populations of vpr+ and vpr- proviruses displayed distinct spectra of responses to latency reactivation agents.

Conclusions: Keying epigenetic features of tissue culture integrants to those observed in patients suggested that the spectra of integration sites observed within experimental vpr+ polyclonal populations were not different from those observed in vivo but that the vpr- proviruses were different, suggesting interclonal variation in expression patterns observed in tissue culture also pertain to primary infection. The selective advantage of clones that seldom express virus, paired with little to no disadvantage associated with high burst size, may contribute to proviral persistence properties and to patients' blips of viremia.

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PP 3.6 – 00122

Heterogeneous associations between HIV genomic integrity and proviral longevity during long-term ART

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Background: Understanding proviral longevity is critical to HIV remission strategies, but previous phylogenetic studies inferring within-host proviral ages have not differentiated between intact and defective viral genomes. We characterized the ages of intact and defective proviruses,ex vivo reactivated proviruses, along with plasma HIV sequences from post-ART rebound and on-ART low-level viremia events, in six participants who had received ART for a median 9.5 years.

Methods: We used single-template-sequencing to longitudinally characterize evolving plasma HIV (nef) populations over a median of 7 years pre-ART, along with near-full-genomes of HIV sequences persisting on ART. To mitigate uncertainty in within-host phylogenetic reconstruction, we inferred a median 1,500 phylogenies per participant from nefsequence alignments using Bayesian approaches. We calculated host-specific pre-ART HIV evolutionary rates from these trees, which were then used to convert root-to-tip distances of on-ART sequences to their integration dates.

Results: We collected 885 pre-ART nef sequences and nearly 2500 on-ART HIV genomes (overall 4% genomically intact; 30% with intact nef). Proviruses with large deletions dominated 5 of 6 participants' on-ART proviral landscapes, where we observed numerous examples of unique proviruses sharing exact deletion "breakpoints" both within and across participants. Clonal sequences, ranging from 17 to 55% within-host frequency, were enriched among intact and psi-defective proviruses, but not proviruses with large deletions or hypermutation, suggesting clonal expansion is a less critical enabler of longevity for the latter proviruses, which instead possess an inherent persistence advantage through decreased likelihood of HIV expression. While all participants' on-ART proviral pools contained at least some sequences dating to earlier periods of untreated infection, all of these longest-lived proviruses were defective, while all intact proviruses and sequences from ex vivo and in vivo viral rebound events dated to near ART initiation. Intriguingly, on-ART low-level viremia was sometimes fueled by very old within-host proviral sequences, with phylogenies suggesting a potential defective proviral origin in some cases.

Conclusions: Results strongly suggest genomic integrity reduces proviral longevity. Nevertheless, further investigation of the anatomic/cellular sources of low-level viremia is needed before firmly concluding that HIV remission strategies need only

focus on eliminating the smaller subset of genetically younger proviruses.

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PP 3.7 – 00128

Genetic variation of the HIV-1 subtype C transmitted/founder viruses long terminal repeat elements and the impact on transcription activation potential and clinical disease outcomes

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Background: Genetic bottleneck is a hallmark of HIV-1 transmission such that only a limited number of viral variants, termed transmitted/founder (T/F) variants are transmitted or establish infection in a newly infected host. The phenotypic characteristics of these variants may determine the subsequent course of disease. The HIV-1 5′ long terminal repeat (LTR) promoter drives viral gene transcription. We hypothesized that HIV-1 subtype C (HIV-1C) T/F virus LTR genetic variation is a determinant of transcriptional activation potential and clinical disease outcome.

Methods: The LTR was amplified from plasma samples of 41 study participants acutely infected with HIV-1C (Fiebig stages I and V/VI). Paired longitudinal samples were also available at one year post-infection for 31 of the 41 participants. LTR amplicons were cloned into a pGL3-basic luciferase expression vector, and transfected alone or together with Transactivator of transcription (Tat) into Jurkat cells in the absence or presence of cell activators (TNF-α, PMA, Prostratin and SAHA).

Results: Inter-patient T/F LTR sequence variation with subsequent intrahost viral evolution observed in 36.6% of the participants analyzed at 12 months post-infection. T/F LTR variants exhibited differential basal transcriptional activity, with significantly higher Tat-mediated transcriptional activity compared to basal ($p < 0.001$). Basal and Tat-mediated T/F LTR transcriptional activity showed a significant positive correlation with contemporaneous viral loads and negative correlation with CD4 counts ($p < 0.05$) respectively. Furthermore, Tat-mediated T/F LTR transcriptional activity showed a significant positive correlation with the viral load set points, viral loads but negatively with CD4 counts at one year post infection (all $p < 0.05$). Lastly, PMA, TNF-α and SAHA cell stimulation resulted in enhanced yet heterologous response of different T/F LTR variants.

Conclusions: Our data suggest that T/F LTR variants may influence viral transcriptional activity, disease outcomes and sensitivity to cell activation. Future studies should investigate the impact of T/F LTR genetic variation on latency development and/or reactivation.

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PP 3.8 – 00137

Characterization of the HIV-1 subtype C reservoir during ART in South-African men and women

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Background: Subtype C is the predominant HIV subtype, nonetheless subtype B is most studied. To develop a global HIV cure strategy it is important to characterize subtype C viral dynamics and reservoir. The biggest obstacle to a cure is the proviral DNA reservoir, consisting of an intact and defective fraction. The aim of this study was to investigate the impact of late presentation (low CD4-count) and gender differences on size and activity of subtype C intact and defective viral reservoir in people with HIV in rural South Africa.

Methods: 78 participants were included (65%female). Median CD4-count at antiretroviral therapy (ART) initiation was 266.5 cells/mm3 and median viral load (VL) 48,652 copies/mL. Patients started protocolized first-line ART. At baseline and 48 weeks PBMCs were collected for analyses of intact and defective proviral DNA (IPDA) and msRNA as a measure of reservoir activity.

Results: At baseline a significantly higher CD4-count was observed for females compared to males ($p < 0.05$). However, no significant difference in the intact or defective DNA reservoir, msRNA or VL was observed between genders. Significant correlations were observed between baseline VL and msRNA, VL and intact ($P < 0.05$), and defective proviral DNA ($P < 0.01$). Inverse significant correlations were found between VL and CD4, and CD4 and msRNA ($P < 0.01$). Interestingly, msRNA and CD4 were significant, positive and inverse correlated respectively, with defective ($P < 0.01$ and $P < 0.05$ resp.) but not with intact proviral DNA at baseline. During treatment, a significant decrease of both the intact and defective reservoir, as well as msRNA was detected ($P < 0.01$). The decrease of the intact reservoir was significantly more profound than the decrease of the defective proviral DNA ($P < 0.05$).

Conclusions: Decrease of the HIV reservoir size during treatment is similar between genders, with a more pronounced decline of the intact reservoir and no impact of late presentation on reservoir size at baseline. Interestingly, levels of msRNA correlate with the size of the large defective viral reservoir rather than the smaller intact reservoir demonstrating that the defective reservoir actively transcribes msRNA. A combination of IPDA and msRNA could form the basis for the evaluation of HIV cure interventions.

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PP 3.9 – 00146

Impact of time on antiretroviral therapy on the proviral reservoir in people living with HIV

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Background: Several studies have shown that intact proviruses decline rapidly after antiretroviral therapy (ART) initiation and continue to decline after 7 years of ART. This observation

suggests that most of the cells harboring intact proviruses may have been cleared in individuals treated for prolonged periods of time. To determine whether long-term treated individuals may interrupt treatment without the risk for viral rebound, we assessed the composition of the viral reservoir in people living with HIV (PLWH) on prolonged ART without any measured viral blips.

Methods: Leukapheresis was performed on five PLWH receiving ART for over 20 years with no registered viral blips (average time before ART = 5.0 years, average time on ART = 27.5 years) and a reference population of 14 PLWH receiving ART for a shorter time (average time before ART = 4.1 years, average time on ART = 11.8 years). Viral reservoir size in CD4+ T cells was quantified by digital PCR combining total HIV DNA and intact proviral DNA assay (IPDA). The viral composition was assessed by a long-read sequencing assay developed in-house which enables high-throughput characterization of the proviral HIV landscape.

Results: Long-term treated individuals had a low reservoir size with a median of 374 HIV DNA copies/million CD4+ T cells (95% CI [339,408]) compared to the reference population with a median of 1340 HIV DNA copies/million CD4+ T cells (95% CI [1429,1251]). All long-term treated PLWH had a low but detectable number of intact proviruses measured by IPDA (5.47 intact proviruses/million CD4+ T cells (95% CI [0.74, 10.21]). Using long-read sequencing, 101 curated distinct proviruses were detected of which 12 were clonally expanded (average 2.4 clones per individual). Only three distinct intact proviruses were observed in 3 of out 5 individuals, of which one provirus belonged to a clone.

Conclusions: Smaller reservoir was detected in these long-term ART-treated individuals compared to individuals treated for a shorter period of time. Despite prolonged antiretroviral therapy, remaining intact proviral sequences were detected by digital PCR in all participants and in 3 out of 5 PLHW with long-read sequencing. Ongoing experiments should provide further insight on the inducibility of the remaining intact proviruses.

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PP 3.10 – 00168

Vpr synergizes with vorinostat to prevent HIV-1 latency establishment

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Background: The long-lived reservoir of cells latently infected with HIV-1 presents a significant barrier to cure. Although some latency-reversing agents induce viral gene transcription, none alone have reduced reservoir size. Recent research suggests that latency prevention may be a more effective approach. Our preliminary work shows that vorinostat (VOR), a histone deacetylase inhibitor (HDACi), prevents latency establishment in a primary CD4+ T-cell model. These findings suggest that HDACs play a critical role in establishing latency and represent targets for latency preventing agents (LPAs). However, it is unclear whether HDACs interact with specific viral factors to regulate latency establishment. Notably, the HIV-1 accessory protein Vpr is a viral gene co-activator. We therefore sought to study how both Vpr alone and the combined activity of Vpr and HDACis affect HIV-1 latency establishment.

Methods: We infected activated primary $CD4^+$ T cells with GFP-expressing reporter HIV-1 strains with or without Vpr and treated with VOR or vehicle control. Flow cytometry was used to measure GFP-expressing cells percentage and median GFP expression weekly for 3 weeks.

Results: Vpr expression had little effect on HIV-1 silencing in $CD4^+$ T cells, suggesting that latency is largely Vpr-independent. Interestingly, however, Vpr expression resulted in an increase in central memory T cells (TCM) in the culture. Further, VOR-treated cells infected with Vpr-expressing virus had significantly higher viral gene expression compared with VOR-treated or Vpr-expressing cells alone.

Conclusions: Our findings demonstrate that Vpr promotes increased abundance of TCM cells in a latency model. This finding is of note because the latent reservoir is enriched in these longer-lived cell types. In addition, our data suggest that Vpr synergizes with HDACis in promoting viral gene expression and preventing latency establishment. A better understanding of how host and viral factors interact to regulate latency establishment will facilitate candidate LPA identification.

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PP 3.11 – 00171

The chromatin insulator CTCF inhibits HIV gene expression

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Background: The long-lived, latent HIV reservoir that persists during HIV antiretroviral therapy (ART) is a significant hurdle in the goal to cure HIV. During HIV infection, proviruses becomes transcriptionally silent by mechanisms that are yet to be well defined. To address this, we characterized a primary cell model of HIV latency that results in CD4 T cell populations that have active and latent HIV transcription. In this model we observe that HIV transcriptional latency was stable and heritable during cell division.

Methods: Assay of Transposon-Accessible Chromatin sequencing (ATACseq), HOMER tool, Cleavage Under Targets and Release using Nuclease (CUT&RUN), CRISPR KO.

Results: By using the Assay of Transposon-Accessible Chromatin sequencing (ATACseq), we observed a significant reduction in HIV proviral accessibility during latency. Additionally, we utilized HOMER, a transcription factor (TF) motif enrichment tool, to identify possible transcription factors (TFs) that can be binding to the differentially open peaks of latent and active HIV cells. Sites for the chromatin insulator CTCF were highly enriched in the differentially open chromatin of infected

CD4 T cells. To test the functional role of CTCF in HIV infected cells, we depleted CTCF expression in both cell line and primary cell models of latency. Upon CTCF depletion, HIV gene expression was reactivated in latently infected cells, highlighting CTCF as a novel regulator of HIV latency. Furthermore, by using Cleavage Under Targets and Release using Nuclease (CUT&RUN) we discovered that CTCF binds to the LTR regions of the HIV provirus.

Conclusions: These data identify CTCF as an important new regulator of HIV gene expression that could be targeted to reverse latency in addition to a new non canonical binding site in the provirus for CTCF.

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PP 3.12 – 00180

Distinct HIV-1 resistance profiles against bNAb in intact vs defective viral genomes

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Background: Broadly neutralizing antibodies (bNAbs) targeting HIV-1 Env are under study as a strategy in cure research. Viral *env* is genetically diverse, with distinct bNAb sensitivity profiles across subtypes and across hosts. We analyzed and identified bNAb-associated resistance genotypes in intact and defective HIV-1 DNA genomes and hypothesize that intact genomes will have distinct bNAb resistance profiles relative to defectives.

Methods: HIV-1 DNA genomes were sequenced from 1 viremic and 14 virologically suppressed individuals on long-term ART via single genome amplification (HXB2 638–9632). Genomes were classified as intact or defective using HIVSeqinR software. Resistance residues against CD4 binding site-, V2-, and V3-targeting bNAbs were examined, as well as hypervariable loop characteristics such as total length, number of N-linked glycosylation sites (PNGs), and electric charge were assessed.

Results: All 15 study donors displayed distinct resistance profiles against CD4 binding site-, V2- and V3-targeting bNAbs (Figure 1A). In-depth analyses of two virologically suppressed donors revealed distinct profiles: Donor 1 (D1) had overall the lowest diversity among donors with >2 sequences (0.4% compared to the 1.2% average pairwise diversity), and this reflected in very similar resistance profiles in intact compared to the defective genome *envs* (Figure 1A). In contrast, Donor 3 (D3) defectives genomes *envs* had higher diversity compared to intact genome *envs*, and had significantly more resistant residues (Figure 1B, all $p < 0.0006$), significantly longer V1 + V2 hypervariable loops ($p = 0.005$), and were more negatively charged ($p = 0.002$). Two glycans, at positions 289 and 743, both associated with increased V3 bNAb resistance, were exclusively found in defective genomes. In the viremic donor (D7), 91% defective viruses lacked *env*, whereas the 20 intact genomes had two distinct bNAb resistance profiles, equally split, one with significantly more resistant residues, as well as longer V1 + V2 loop lengths and a higher number of PNGs ($p < 0.004$) – all characteristics associated with higher V3 bNAb resistance (Bricault et al., 2019).

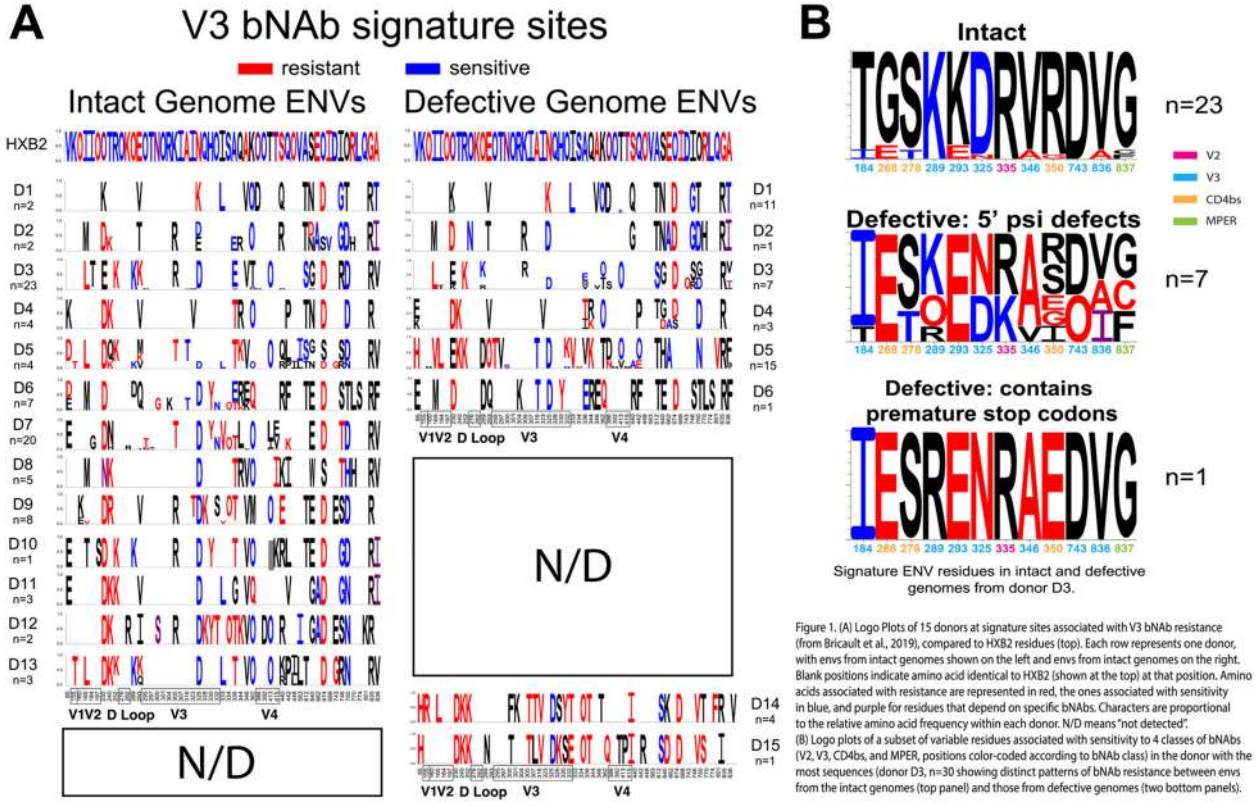

Figure 1. (A) Logo Plots of 15 donors at signature sites associated with V3 bNAb resistance (from Bricault et al., 2019), compared to HXB2 residues (top). Each row represents one donor, with envs from intact genomes shown on the left and envs from intact genomes on the right. Blank positions indicate amino acid identical to HXB2 (shown at the top) at that position. Amino acids associated with resistance are represented in red, the ones associated with sensitivity in blue, and purple for residues that depend on specific bNAbs. Characters are proportional to the relative amino acid frequency within each donor. N/D means "not detected". (B) Logo plots of a subset of variable residues associated with sensitivity to 4 classes of bNAbs (V2, V3, CD4bs, and MPER, positions color-coded according to bNAb class) in the donor with the most sequences (donor D3, n=30 showing distinct patterns of bNAb resistance between envs from the intact genomes (top panel) and those from defective genomes (two bottom panels).

Figure 1 (abstract: PP 3.12–00180) HIV-1 resistance profiles against bNAb.

Conclusions: Our results reveal that resistance profiles across all bNAb classes in *env* region are diverse across hosts and distinct between intact and defective genomes within host, and highlight the importance of understanding intact-genomes resistance profiles when screening individuals for enrollment in bNAbs clinical trials.

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HIV-1-infected individuals with extremely low reservoir under ART are characterized by reduced viral diversity and higher levels of hypermutations in their viral reservoirs

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Background: HIV cure strategies aim to eliminate viral reservoirs that persist despite successful antiretroviral therapy (ART). LoViReTs are a group of HIV infected individuals that are characterized by very low levels of provirus during ART treatment. In the present study, we have studied the genetic characteristics of their blood viral reservoirs.

Methods: We amplified and deep-sequenced regions of gag, pol, and env from the HIV viral populations in blood reservoirs during ART from 22 LoViReTs and compared them with 22 matched controls defined both as < and >100 HIV-DNA copies/106 CD4+ T cells during ART respectively. Additionally, we were able to analyze matched samples from some of the patients prior to therapy. We reconstructed viral consensus sequences and quasispecies haplotypes from each sample. We analyzed viral diversity and genetic characteristics of the viral populations, and examined associations between viral features and host factors comparing LoViReTs with controls.

Results: Our analyses indicate a decreased viral diversity detectable in env gene when comparing LoViReTs with controls (p-value = 0.036). This result was even more significant when fitting a multivariable model to control for time from diagnosis and time of viral suppression (p-value = 0.027). When pre-ART sequences were analyzed, despite the low sample size, we could observe a trend indicating a lower viral diversity already present in LoViReTs before ART. These differences were not due to any host factor analyzed and were attained despite similar average number of virological failures, as well as levels and number of viremia peaks or bleeps during ART between both groups. Additionally, LoViReTs displayed a significantly higher number of mutations in APOBEC sites in the pol gene within their quasispecies (p-value < 0.001), indicating a high degree of deleterious viruses produced during ART.

Conclusions: The group of LoViReTs studied maintained a significantly less diverse viral reservoir despite similar pre-ART viral load and similar number of therapy failures during ART. This lower variability is also associated with higher levels of hypermutated mutants in the reservoirs of these patients. A decreased reservoir diversity, possibly before therapy, could explain the smaller viral reservoir observed in LoViReTs during ART.

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PP 3.14 – 00190

Ex vivo response to latency reversal agents of CD4+ T cell subsets and monocyte derived HIV-1 subtype C LTR from individuals on suppressive cART

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Background: The HIV-1 latent reservoir is primarily established early in infection in CD4+ T cell memory subsets, with emerging findings supporting the establishment of latency in monocyte/macrophage lineage cells and naïve CD4+ T cells. Latency reversal agents such as HDAC inhibitors (HDACi's) reactivate viral transcription by targeting the LTR, which results in a variable reactivation potential. The cause of the variable response has not been well elucidated. Therefore, we aim to characterize the genetic variation of cell subsets (CD4+ T naïve, central, effector, transitional memory, and monocyte) derived proviral HIV-1 subtype C (HIV-1C) LTR and assess their sensitivity to HDACi's. We hypothesize that cell subset specific LTR genetic variation may translate to differential functional activity and responses to HDACi's.

Methods: CD4+ T naïve, memory cell subsets and monocytes were cell sorted from blood mononuclear cells (PBMCs) from four HIV-infected South African females. From each cell subset, the 5′ LTR was amplified, cloned, and sequenced. Subsequently, we performed a transcriptional assay to assess the functionality of these cell subset derived 5′LTR and assessed their sensitivity to reactivation using HDACi's.

Results: Phylogenetic analysis of the full-length HIV-1 5′LTRs bulk sequences derived from cell subsets comprising the latent reservoir clustered together per patient. There were no significant branch length differences of the 5′LTR sequences at transmission, prior to or following treatment within a cell subset indicating no 5′LTR evolution. Particularly the transcription factor binding sites were conserved. Our data support the findings that latent reservoir is seeded early by homogenous transmitted/founder virus. Furthermore, the cell subset derived 5′ LTR were functional, with enhanced tat induced transcription compared to basal transcription. Lastly, the cell subset derived 5′ LTR were sensitive to activation by HDACi (Trichostatin A, Panobinostat and Vorinostat), with Trichostatin A demonstrating the highest activation potential, albeit a heterologous response was observed between subsets.

Conclusions: These findings inform future studies that a targeted approach is needed to achieve virus eradication due to the heterogenous cell population comprising the HIV-1 latent reservoir.

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PP 3.15 – 00207

A polyvalent HIV-1 virus-like particle formulation drives the majority of the infectious HIV-1 reservoir out of latency within CD4+ T cells of individuals receiving cART during chronic infection

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Background: Elimination of the latent viral reservoir is difficult due in part to the lack of surface markers identifying a latently infected cell. A previous study using patients treated during acute infection revealed that potent and targeted latency reversal (LR) was achieved by activating HIV-specific T cell receptors using non-infectious HIV-like particles (ACT-VEC). The current study focuses on LR evaluation in the >95% of patients that receiving cART during chronic infection.

Methods: PBMCs were obtained from 32 infected individuals from Kampala (Uganda), Cleveland, and Toronto after 6 to 20 years of undetectable viral load on cART. ACT-VEC was presented to patient CD4+ T cells using autologous monocyte-derived dendritic cells. LR was measured by viral release in culture and intracellular viral RNA. Nanopore and Illumina sequencing were performed on the released virus following LR to determine viral diversity and infectious:noninfectious virus ratio. IFN-g ELISpot were used to measure T cell activation.

Results: ACT-VEC induced CD4 T cells (from chronic patients on cART) to release 100-fold more HIV-1 into culture supernatant than stimulation with common recall antigens, PMA/Ionomycin or anti-CD3/anti-CD28 antibodies; and 1000-fold more than by HDAC inhibitors, PKC agonist, or TLR7 ligands. Even after 6 to 20 years of treatment, 1000-fold more latent virus was activated/released from CD4+ T cells in patients treated during chronic infection than in those receiving cART for <3 years initiated during acute infection. Subtype B derived ACT-VEC was only slightly (<2-fold) less potent than the subtype D derived ACT-VEC at LR in the CD4+ T cells of subtype D infected Ugandans on cART. HIV-1 released in supernatant with ACT-VEC treatment directly correlated with amount of infectious virus propagated from mitogen activated PBMCs from same patients. Likewise, a high proportion of infectious virus was released by ACT-VEC LR based on Nanopore and Illumina sequencing of genomic viral RNA.

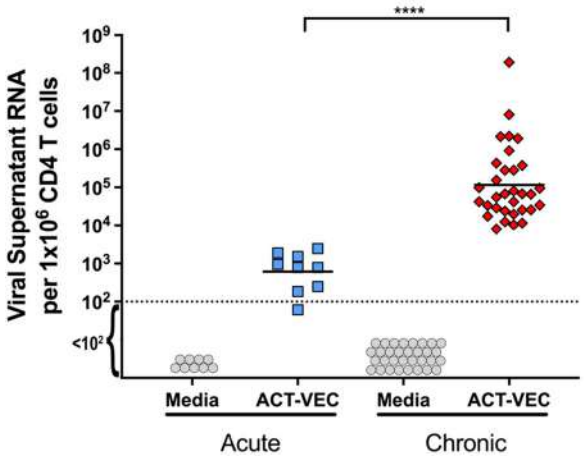

Figure. ACT-VEC latency reversal with acute versus chronic

Conclusions: ACT-VEC is a potent stimulator LR in patients receiving treatment during chronic infection with evidence that infectious HIV-1 reservoir is primarily housed in HIV-specific CD4+ T cells. In both Ugandans and Canadians, an infectious HIV-1 reservoir was readily activated by ACT-VEC even after 20 years of stable cART.

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PP 3.16 – 00209

Prolonged persistence of HIV-infected cells in tissues after allogeneic hematopoietic transplant

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Background: HIV persistence during antiretroviral therapy (ART), prevents HIV eradication or control. Mechanisms responsible for HIV persistence, including in tissues, are not well understood. Here we investigated persistence of HIV-infected cells in tissues for >200 days in an individual who had successful allogeneic hematopoietic cell transplant (Allo-HCT; donor CCR5 wild-type) but developed acute lymphocytic interstitial pneumonia after COVID vaccination and expired.

Methods: Clinical and autopsy records were reviewed. HIV in tissue samples and mononuclear cells (prepared by ficoll from lung and spleen) were studied by single genome sequencing (SGS, 1.1 kb gag) and single-copy PCR for HIV DNA.

Results: Study participant was a 38 yo man with HIV/AIDS on ART with HIV RNA < 50 c/ml for >3 years who had reduced-intensity conditioning, HLA-mismatched unrelated donor allo-HCT for primary refractory ALK-negative anaplastic large cell lymphoma. His course was remarkably uncomplicated:100% engraftment of donor CD3 and myeloid subsets confirmed by day +42 post-HCT; 100% donor cells (CD4, CD8, CD19, CD14, CD56) confirmed by day +100. Per protocol, he was off all immunosuppression on day +60, did not develop acute graft-versus-host disease (GVHD), and maintained ART with HIV RNA < 20 c/ml. Immune reconstitution was robust: NK, CD8,

and B cells within normal range, CD4 = 172 cells/μL at d + 100. He received SARS-CoV2 mRNA vaccine (Moderna) on days +107 and +144. On d + 155, he developed dyspnea, hypoxia with acute lymphocytic interstitial pneumonia, presumed vaccine-related, with no signs of GVHD, pulmonary infection, or lymphoma. HIV-1 RNA remained <20 except 21 c/ml noted shortly before expiring on d + 207. Postmortem notable only for diffuse alveolar damage with fibrosis and a small white pulp-depleted spleen. Single copy assay detected HIV DNA in brain (thalamus, frontal lobe, midbrain), lymph node, and jejunum (1.6–16 copies/106 tissue cells) but not liver, spleen, or affected lung (<0.2 copies/106 cells). SGS revealed HIV sequences were non-identical, demonstrating multiple distinct populations of infected cells were present.

Conclusions: HIV populations are diverse in tissues even after extensive lymphodepleting chemotherapy and allo-HCT. Complete donor engraftment in tissues, including brain, may take significantly longer than engraftment measured in peripheral blood. Eradication strategies will require evaluation of tissue compartments.

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PP 3.17 – 00211

Genetic Diversity of HIV-1 Long Terminal Repeat in Proviral Populations During Long-Term Antiretroviral Therapy

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Background: The HIV proviral populations remain dynamic after long-term antiretroviral therapy (ART) but mechanisms responsible for this are uncertain. During ART, the majority of proviruses are transcriptionally silent, and it is possible that levels of expression from HIV-promoter may contribute to the differential dynamics of intact and defective proviruses. To investigate the effects of long-term ART on HIV promoter sequences, we characterized genetic variability of HIV-LTR in total, intact, and near-full length (NFL) defective proviruses prior to and following long-term ART.

Methods: Peripheral blood lymphocytes from 8 persons living with HIV (PLWH; N = 8) were obtained prior to and following long-term ART (range 4–20 y). Single genome sequences (SGS) of NFL proviruses (>7 kb) were obtained (Illumina MiSeq) and genetic intactness was determined by analysis of open reading frames and regulatory sequences. We also sampled the total proviral LTR population by obtaining SGS of the LTR alone (450 bp). We analyzed LTR sequences using measures of genetic diversity (average pairwise difference, APD) and population structure (phylogenetics, Slatkin-Maddison analyses). We also investigated the predicted promoter activity of LTR transcription factor binding sites (TFBS) based on TRANSFAC database.

Results: We obtained a range of 0–32 intact, 2–32 NFL-defective, and 10–36 total LTR sequences per timepoint. No significant differences were detected in LTR genetic diversity (APD 0.98–1.8%) in total, intact, or NFL-defective proviruses in all comparisons prior to and following long-term ART. From pretherapy to long-term ART, no changes in population structures of intact were observed but significant changes of NFL-defective were observed in 2/8 and of total proviruses in 1/8 PLWH. LTR populations in intact and NFL-defective proviruses were, however, distinct from LTR in the total proviruses in 5/8 patients. Significant differences between NFL and total proviruses in genetic variations in TFBS were present in a minority of individuals, with changes in CCAAT-box binding (3/8 PLWH), NF-KappaB (3/8), CP2 (2/8), and SP1 (1/8) sites.

Conclusions: HIV-LTR sequences remain diverse in intact and defective proviruses but undergo population shifts from total LTR sequences after long-term ART in the majority of PLWH. Genetic variations in TFBS, which suggest differences in basal transcriptional activities of NFL proviruses may be present in some PLWH.

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PP 3.18 – 00027

Role of Tunneling Nanotubes-like Structures during the Early Events of HIV Infection and viral reactivation

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Background/Aims: The HIV pandemic affects 38.5 million worldwide and is still ongoing despite the successful introduction of ART. However, ART is not a cure due to the early generation of viral reservoirs (VRs). Despite all the efforts to eradicate these latently infected cells, their elimination has not been achieved. A critical question is how few VRs can repopulate the entire body in a few weeks post ART intervention. Most viral amplification models indicate that soluble virus is not sufficient to explain reactivation. The identification of the virological synapsis provided an alternative mechanism of cell-to-cell infection, but still, this area of research is unknown. We identified that tunneling nanotubes, TNTs, could provide an additional mechanism of cell-to-cell transmission. Our hypothesis is that HIV induces TNTs, the viral spread from HIV latently infected cells to uninfected cells.

Methods: To characterize TNTs formation between uninfected and HIV-infected cells, we developed a co-culture system using human primary macrophages. Live-cell imaging and confocal microscopy were used to evaluate TNT formation and material transfer. We also used microinjection to examine direct cell-to-cell infection. We performed live-cell imaging microscopy experiments using transfected cells with Gag YFP plasmids to identify if the transfer involves a Gag-mRNA or Gag protein complex.

Results: Our results showed that HIV infection induced the formation of TNTs between HIV-infected and uninfected cells. TNTs allow the transfer of infectious agents from HIV-infected cells to uninfected cells. TNTs induction with H_2O_2 and subsequent microinjection of a mature virus, or HIV viral proteins did not result in TNT mediated transmission, except gag, suggesting that TNT is highly selective to the transported cargo.

Conclusion: HIV-infected cells can form TNTs to spread infection; our data showed that TNT-connected cells transfer several HIV components. TNTs are a potential target to prevent viral reactivation by reducing the transfer of infectious/damaging pathogenic mediators. We propose that TNTs are a therapeutic pathway to eliminate VRs.

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PP 3.19 – 00062
The Role of Pannexin-1 channels in HIV infection and persistence
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Background/Aims: Human immunodeficiency virus-1 (HIV) infection of the CNS can have devastating neurological consequences, but the cellular and molecular basis of HIV CNS dysfunction is still poorly understood. Our lab recently identified a novel host protein involved in both acute and chronic HIV infection and NeuroHIV, Pannexin-1 (Panx-1). Panx-1 channels are large ionic channels that remain in a closed state in healthy conditions. In pathological conditions, such as acute and chronic HIV infection, Panx-1 channels open, releasing large signaling molecules into the extracellular space, including ATP. Upon ATP release through the channel, ATP activates autocrine and paracrine purinergic receptors to induce damage.

Methods: Our laboratory has used electrophysiological, imaging, pharmacological/molecular approaches, patient data, and animal model experiments. The experiments conducted used both PBMCs and sera from HIV-infected patients, contrasted to age-matched, uninfected controls.

Results: We identified that Panx-1 channels are extremely active during long-term HIV infection, even in the absence of systemic replication due to ART. We identified that all chronic HIV-infected individuals analyzed had uncontrolled Panx-1 channel opening resulting in high circulating ATP levels despite effective ART. Increased serum ATP levels perfectly correlated with cognitive decline, suggesting that ATP in the circulation can be used as a biomarker of cognitive impairment in the HIV-infected population. We identified that ATP in the HIV-infected population induces accelerated leukocyte differentiation into a migratory phenotype characterized by higher expression of key adhesion and tight junction proteins required for adhesion and transmigration across the Blood-Brain Barrier (BBB).

Conclusion: High ATP compromised the BBB integrity and promoted the transmigration of uninfected and HIV-infected cells across the BBB by a Panx-1, ATP, and purinergic-dependent mechanism. We propose that HIV dysregulation of Panx-1 channels is essential for the chronic effects of HIV toxicity and persistence.

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PP 3.20 – 00154
Epigenetic modifying compounds negatively impact viral replication within primary human macrophages
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Background/Aims: Antiretroviral therapy (ART) can suppress HIV replication, unfortunately this therapy is not curative. ART must be taken for life because cessation of treatment results in viral rebound. This can be explained by the persistence of the virus in different cell type including macrophages (Jambo *et al.*, Mucosal Immunol., 2014). In contrast to T lymphocytes, these phagocytes are resistant to the cytotoxic effect of the virus (Boliar *et al.*, PNAS, 2019) and have a long lifespan (from months to years). Understanding how HIV replicates and is maintained in these immune cells may provide the means to eliminate this reservoir.

Methods: To answer to this question, we perturbed host cell physiology with a panel of 735 epigenetic modifying compounds. To assess viral production, we constructed a sensitive Gaussia luciferase HIV reporter, NL4-3 BaL IRES-g*Luciferase* (HIV-Gluc). After 6 days of differentiation, we infected human monocyte-derived macrophages (hMDMs) *in vitro* with HIV-Gluc. One day after infection, the cells are treated with epigenetic modifiers and 6 days later, Gaussia luciferase released into the supernatant was quantified by plate reader.

Results: The screening revealed that compounds known to inhibit of KDM4C (Lysine Demethylase 4C) or Sirtuins decreased Gaussia release by hMDMs. Thus, these epigenetic modulating agents decreased the viral production and these results were confirmed by flow cytometry. qPCR analysis demonstrated that these small molecules induced a decrease of viral RNA and DNA inside the cells. We are currently investigating the mechanisms by which these compounds reduce viral production.

Conclusion: The results demonstrate that perturbation of KDM4C and Sirtuins negatively impact the viral cycle in primary macrophages, at the level of the reverse transcription and/or DNA integration.

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Session 4: Immunology of HIV Persistence

OP 4.1

Viral persistence and NK cells

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Natural killer (NK) cells serve on the front line of immunity, mounting immediate and powerful defenses against cells displaying molecular signals of stress, transformation, or infection. NK cell activity is tightly controlled through the balance of a large repertoire of inhibitory and activating receptors, and it is the unique combination of these receptors expressed by individual cells that confers immense diversity both in phenotype and functionality. This diversity depends not only on genetic factors but is more plastic as previously expected and is modulated by extrinsic parameters such as cytokine and tissue-specific environments, previous infections and vaccinations, age and way of living (smoking, obesity, etc.). The recent discoveries of the capacity for NK cells to adapt and also to differentiate into long-lived antigen-specific memory cells has added further complexity to this field, while raising novel opportunities for harnessing these cells. A role of NK cells in the control of viral replication in HIV-1 infection has been demonstrated for a long time, for instance through studies on HLA-I alleles. NK cells have the potential to directly impact HIV reservoirs. However, HIV-1 infection most often diminishes NK cell antiviral effector functions, increases their inflammatory state and pathologically changes their tissue distribution diminishing their capacity to control viral reservoirs. Only few studies analysed the function of NK cells in tissues during HIV infection. By analysing NK cells in tissues in animal models we provided novel insights into mechanisms regulating their distribution within tissues, their role in viral reservoir control and factors that can potentially enhance their antibody-dependent cytotoxic cellular (ADCC) activity. While the role of NK cells has been well appreciated in the early phase of infection, adaptive NK cells might provide additional relevant effectors for long-term viral reservoir control. We will report common and distinct features of adaptive NK cells in SIV and other viral infections. Altogether, the recent insights into NK cell biology provides further means to tackle viral reservoirs

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OP 4.2 – 00085

Cytolytic CD8+ T cells infiltrate germinal centers and limit HIV replication in spontaneous controllers

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Background: HIV infection persists predominantly within follicular helper CD4+ T cell-rich B cell follicles of lymphoid tissues. Cytotoxic CD8+ T cells, which are associated with natural control of HIV infection in peripheral blood, are relatively excluded from this niche, representing a potential barrier to cellular immunity and HIV cure. To better understand the mechanisms of HIV control within lymph nodes (LN), we investigated functionality, clonotypic compartmentalization, spatial localization, phenotypic characteristics and transcriptional profiles of LN-resident virus-specific and CXCR5-expressing follicular CD8+ T cells (fCD8) in persons who control HIV without medications.

Methods: We obtained paired excisional inguinal LN biopsies and peripheral blood (PB) from 19 spontaneous HIV controllers and 17 HIV+ individuals on long-term ART. HIV-specific CD8+ T cell responses were identified by IFN-γ ELISpot and functional response to antigenic stimulation was measured by flow cytometry and CFSE-based proliferation assay. Clonotypic compartmentalization and transcriptional signatures associated with localization of HIV-specific CD8+ T cells were assessed via TCR and RNA-sequencing. Spatial relationships between ongoing viral replication and fCD8 cytotoxic effector potential in GCs were measured by HIV gagpol RNAscope and immunofluorescence on fixed LN sections.

Results: Antigen-induced HIV-specific CD8+ T cell proliferation and cytolytic effector upregulation consistently distinguished spontaneous controllers from noncontrollers in PB ($p = 0.03$) and LN ($p = 0.04$). HIV-specific CD8+ T cells from both compartments shared TCR clonotypic composition (Morisita-Horn Similarity Index 0.8–1.0), consistent with ongoing infiltration from circulation. Migration into LNs was associated with gene signatures of inflammatory chemotaxis and antigen-induced effector function. The cytolytic effectors perforin and granzyme B were elevated among virus-specific CXCR5+ fCD8 s ($p < 0.001$). During spontaneous control of viremia, perforin and granzyme B expression by fCD8 s was associated with ongoing HIV replication in GCs ($r = 0.53$, $p < 0.0001$) and proximity to HIV RNA+ cells ($r = 0.85$, $p < 0.001$).

Conclusions: These results identify inflammatory recruitment, antigen-specific proliferation and cytotoxicity of fCD8 s as key features of durable immune control of lymphotropic infection, supporting a cytolytic mechanism by which fCD8 s contain HIV replication in LNs during spontaneous control of viremia. Furthermore, our results highlight that both reduced access to germinal centers and impaired cytotoxic potential of HIV-specific CD8+ T cells represent important barriers to HIV cure in noncontrollers.

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OP 4.3 – 00112

Leaky reservoirs are associated with HIV-specific cd4 and CD8 T-cell responses

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Background: A major obstacle to an HIV cure is the persistence of latent viral reservoirs. When ART is interrupted, latently-infected cells revert to a state of productive infection fueling viral rebound. New sensitive assays suggest that most latently infected cells maintain a certain degree of transcriptional "leakiness," although the in vivo relevance of this phenomenon remains unclear.

Methods: We studied leaky latency in 18 people living with HIV on ART ≥ 3 years (ART-PLWH) with optimal viral suppression. Purified CD4 T cells were either PMA/ionomycin-stimulated (inducible) or unstimulated (leaky) for 16 h. Transcriptionally active (vRNA+) viral reservoirs were identified by single-cell flow cytometric fluorescent in situ RNA hybridization (RNAflow-FISH) using probes targeting LTR-gag, gag or pol regions. Leaky and inducible reservoirs were correlated with HIV-specific CD4+ and CD8+ T cells, as measured by activation induced marker (AIM) and intracellular cytokine staining (ICS) assays. PBMCs were stimulated with Gag, Pol, Env and Nef peptides.

Results: Leaky vRNA+ cells were detected in 14/18 (78%) ART-PLWH samples, compared to 16/18 (89%) for PMA/iono-induced vRNA+. We measured a median of 21 leaky vRNA+ /106 CD4 T cells. Transcriptionally active reservoirs represented 30% of the inducible vRNA+ (median 62/106 CD4, and 4% of the cells containing integrated HIV DNA (median 686/106 CD4). Frequencies of transcriptionally active cells were associated with total, Pol, Env and Nef-specific CD4 T cell responses as measured by AIM (total: $r = 0.532$, $p < 0.05$). A trend was observed for Gag-specific CD4 T responses. For CD8 T cells, although only Env-specific responses reached significance ($r = 0.510$, $p < 0.05$). Similar correlations were observed with ICS. The presence of rare spontaneous p24-expressing CD4 T cells strongly correlated with HIV-specific CD4 T cell responses ($r = 0.640$, $p < 0.01$).

Conclusions: Therefore, leaky latency is detectable in a majority of ART-PLWH, and at frequencies only three times lower than the inducible reservoirs. The correlations between leaky HIV reservoirs and antigen-specific CD4 and CD8 T cells suggest that residual HIV protein expression is a major driver of sustained HIV-specific CD4 and CD8 T cell responses during suppressive ART.

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OP 4.4 – 00018

HIV reservoir burden associates with numbers of HIV-specific CD8+ T cells under long-term antiretroviral therapy and prevents them from differentiating into functional memory cells

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Background: HIV-specific $CD8^+$ T cells play an important role in controlling viral load but become dysfunctional during chronic HIV infection (CHI). Antiretroviral therapy (ART) partially normalizes this defect but residual dysfunction persists when compared to spontaneous controllers and people treated during acute HIV infection (AHI). We hypothesized that the HIV reservoir persisting on ART contributes to this residual dysfunction and prevents the differentiation of HIV-specific $CD8^+$ T cells into functional memory cells under ART.

Methods: We measured HIV DNA levels (LTR-gag) in peripheral $CD4^+$ T cell from people on suppressive ART for more than 1.5 years after initiating ART during AHI or CHI. We analyzed the phenotype of HIV-specific $CD8^+$ T cells recognizing Nef, Pol, Gag, and Env using pMHC tetramers at the same time point on ART.

Results: Both the number and frequency of HIV-specific $CD8^+$ T cells on ART positively associated with the levels of HIV DNA ($r = 0.57$ $P = 0.0003$ and $r = 0.57$ $P = 0.0002$, respectively). In contrast, no association was found between CD8 responses and pre-ART viral load or pre-ART HIV DNA levels, suggesting that HIV-specific $CD8^+$ T cells are responding to the amount of antigen produced from the viral reservoir during ART. Expression of the exhaustion marker PD-1 and the long-lived T cell marker IL-7R on HIV-specific $CD8^+$ T cells associated positively and negatively, respectively, with total HIV DNA during ART ($r = 0.61$ $P = 0.0004$ and $r = -0.63$ $P = 0.0001$). Moreover, frequency of $TCF\text{-}1^{low}TOX^{high}$ exhausted cells and $TCF\text{-}1^{high}TOX^{low}$ stem-like memory/progenitor exhausted cells in HIV-specific $CD8^+$ T cells correlated positively and negatively, respectively, with the HIV reservoir size on ART ($r = 0.62$ $P = 0.0001$ and $r = -0.64$ $P < 0.0001$). These data suggest that the size of the viral reservoir drives the exhausted phenotype under ART while preventing HIV-specific $CD8^+$ T cell to differentiate into long-lived memory cells.

Conclusions: HIV-specific $CD8^+$ T cell numbers and dysfunctional differentiation state are maintained by residual antigen production from the HIV reservoir during ART. This residual dysfunction driven by the HIV reservoir size on ART could contribute to the lack of viral control after analytical treatment interruption and contribute to previous associations reported between the HIV reservoir size and a faster rebound post ART.

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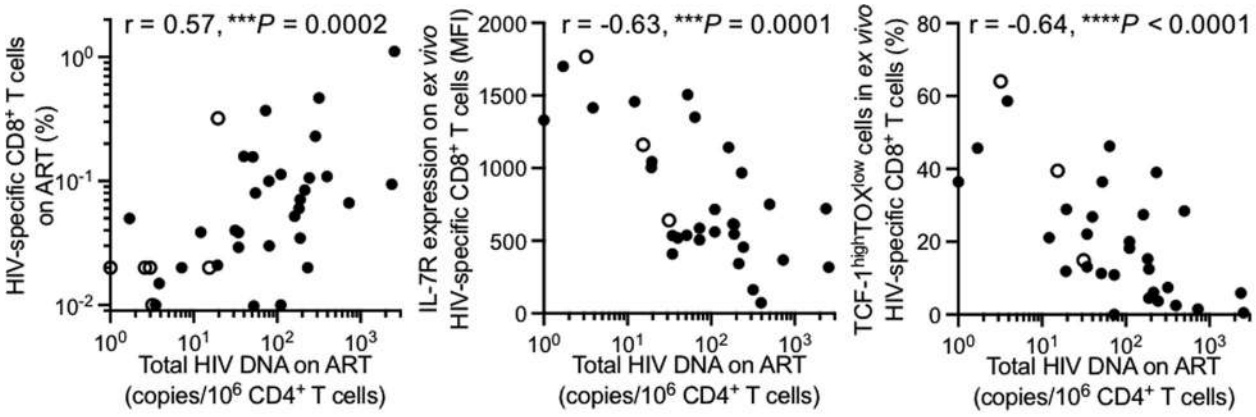

Figure: HIV DNA levels under suppressive ART correlated with frequency of HIV-specific CD8+ T cells, expression of IL-7R and frequency of TCF-1^{high}TOX^{low} on/in HIV-specific CD8+ T cells on ART.

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Figure 1 (abstract: OP 4.4–00018)

OP 4.5 – 00101

Comparative single-cell transcriptome and TCR profiling of HIV infected cells in the blood and cerebrospinal fluid of PLWH before and after ART

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Background: The brain is a site of latently infected cells during HIV infection. However, the dynamics of T cell infection in the central nervous system (CNS) in people living with HIV (PLWH) are incompletely understood.

Methods: We profiled the single-cell transcriptome and T cell receptor (TCR) repertoire of paired blood and cerebrospinal fluid (CSF) CD4 T cells isolated from 7 PLWH and 3 healthy controls. One PLWH was assessed at three longitudinal time points: at time 0 (V1, pre-ART), three (V2) and six (V3) months after starting ART. The other six PLWH were on stable ART. We identified infected (RNA+) single cells by aligning T cell transcriptomes against a consensus HIV-1 sequence. We used Azimuth to annotate cell clusters and Cell Ranger to annotate TCR clonotypes.

Results: We detected a higher frequency of infected single CD4 T cells in CSF than blood (mean 0.055% vs 0.021% for participants on ART, 1.35% vs 0.27% before ART). 84.21% of infected CD4 T cells (before/after ART samples combined) were central memory T cells (Fig 1A, B). We found a reduced level of clonal overlap between blood and CSF after starting ART (Morisita similarity index = 0.44, 0.29, 0.16 for V1, V2, and V3 respectively). There was an increase in repertoire diversity in blood after ART (inverse Simpson index = 211.6, 416.1, 591.7 for V1, V2, and V3 respectively). In CSF, one unique clone was detected only in CSF (not blood) and was expanded in CSF after ART (Fig 1C). Some of the clones that contain infected cells persisted over time in both tissues. In PLWH on ART, we observed lower clonal overlap between blood and CSF than in controls (Fig 1D), and most infected cells were also not clonally related across tissues.

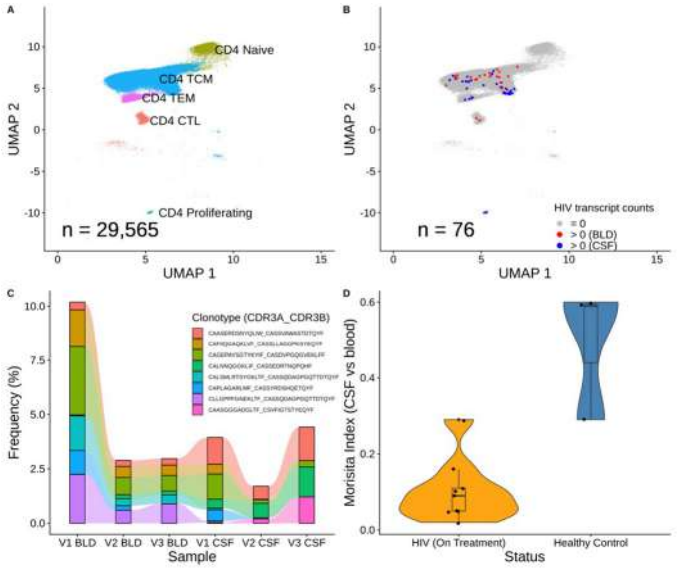

Fig 1. TCR repertoire analysis of PLWH. (A) UMAP of CD4 T cells from blood and CSF, annotated with cell type. (B) UMAP of CD4 T cells with HIV transcripts detected (before/after ART samples combined), with color indicating tissue origin. (C) The frequency of the largest eight clonotypes across three time points in one PLWH (V1 = before ART, V2 and V3 = after ART). (D) Morisita similarity index demonstrating greater overlap in blood and CSF T cell clones in healthy controls compared to PLWH on ART.

Figure. TCR repertoire analysis of PLWH

Conclusions: We find higher frequencies of infected CD4 T cells in the CSF compared to blood. We found expansion of CSF-unique clones after initiation of ART, as well as a divergence of T cell clones in the CSF compared to the blood of PLWH on stable ART. This may reflect ongoing antigen stimulation in the CNS of PLWH despite ART.

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OP 4.6 – 00185

No associations between magnitudes of HIV-specific CTL responses on stable art and subsequent decay of intact proviruses or cell-associated HIV mRNA

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Background: HIV-specific T-cells targeting Nef but not other HIV proteins are associated with levels of total HIV DNA and cell-associated (CA)-RNA that persist on ART, suggesting ongoing stimulation. We hypothesized that decay of intact proviral DNA and CA-RNA levels on ART would be associated with cytotoxic T-cell (CTL) responses.

Methods: 49 participants from the ACTG A5321 cohort on suppressive ART were studied at weeks 24 and 168 post-entry (median 7 years on ART at entry). HIV DNA and CA-RNA were measured by ddPCR (IPDA for DNA, 5′ unspliced and 3′ total poly (A) for RNA). T-cell responses were measured by IFN-γ and granzyme B [GrB] ELISPOT to each gene product (Gag, Env, Pol, Nef, Tat, Rev, and summed HIV). Non-parametric statistics were used to evaluate associations and to compare time points.

Results: 5′ unspliced CA-RNA decreased significantly from week 24 to 168 ($p = 0.001$), and decline in intact ($p = 0.053$) but not defective ($p = 0.22$) HIV DNA approached significance. CA-RNA levels at weeks 24 and 168, and changes from 24 to 168 weeks were not found associated with IPDA levels or changes over time. There were no apparent associations between measures of HIV-specific T-cell responses (both IFN-γ-producing and GrB-producing) with the changes in intact or defective proviruses, nor with the changes in CA-RNA levels – including after controlling for time on ART, pre-ART viral load, and pre-ART CD4 count. As examples, the correlations between magnitudes of IFN-γ-producing Nef-specific responses and changes in intact HIV ($r = -0.11$, $p = 0.61$) and 5′ CA-RNA ($r = 0.06$, $p = 0.71$), or GrB-producing Nef-specific responses and changes in intact HIV ($r = 0.10$, $p = 0.66$) and 5′ CA-RNA ($r = -0.14$, $p = 0.37$) were small and not significant.

Conclusions: While both intact proviral DNA and CA-RNA levels (5′ unspliced) decayed over the 144-week period, contrary to our primary hypothesis no associations were observed between decay of intact HIV DNA or CA-RNA with HIV-specific T-cell responses, including with cytotoxic function (GrB) and despite controlling for time on ART. These findings suggest either a limited role for CTLs in reservoir decay after multiple years of suppressive ART, or that other unmeasured parameters are important, such as variation in susceptibility of reservoir cells to CTL-mediated killing.

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OP 4.7 – 00121

Circulating immune predictors of intact HIV reservoir decay during long-term ART

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Background: The HIV reservoir is not stable during antiretroviral therapy (ART). Cells harboring intact genomes decay more rapidly than those with defective genomes, particularly during the first several years of therapy. The host factors associated with the rate of decay have not been characterized.

Methods: We measured intact proviruses in PWH on ART using the intact proviral DNA assay (IPDA) in peripheral blood. We used the Luminex bead-based multiplexed immunoassay system to measure 32 pro-inflammatory and regulatory cytokines in plasma. Based on our previous study focused on total and intact HIV genome kinetics, we fit linear spline models with a knot at seven years and a random intercept and slope up to the knot. We estimated the influence of baseline cytokine levels and their trajectories on intact HIV kinetics in separate models.

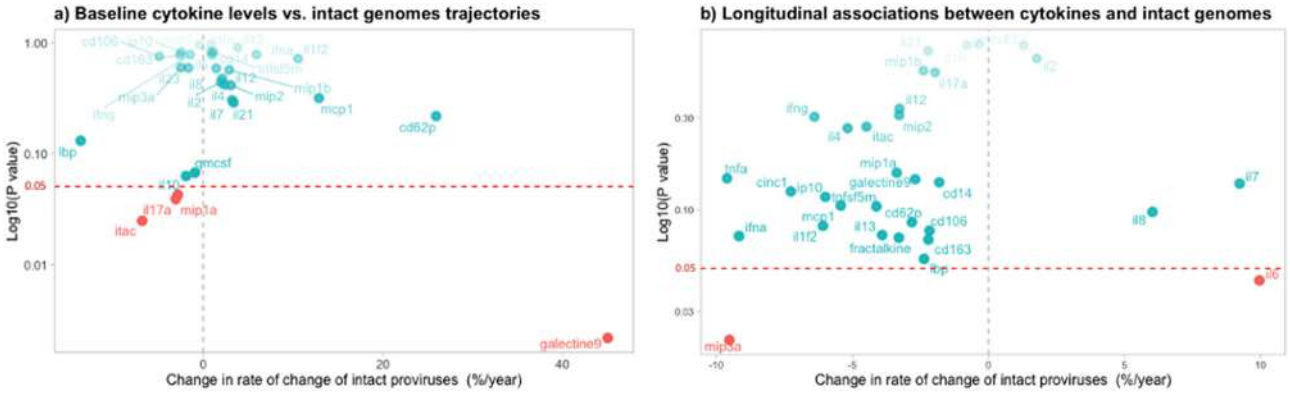

Figure 1 (abstract: OP 4.7–00121) Cytokines and intact HIV kinetics.

Results: We studied 76 PWH on suppressive ART for a median of 10.4 (range 4.3–15.6) years, providing a median of 3 (2–4) samples during the follow-up. Their median nadir CD4 was 180 (0–644) cells/uL and median baseline CD4 count was 591 cells/uL (173–1600). Baseline galectin-9 was the most predictive marker of intact HIV kinetics: per each 10-fold decrease at baseline, there was a mean 45% greater reduction of intact HIV genomes per year ($p = 0.0021$; after adjustment for CD4 nadir, $p = 0.005$). In contrast, lower baseline ITAC, IL-17, and MIP-1a were predictive of intact HIV increases (Figure 1a). MIP-3a and IL-6 exhibited the strongest associations between longitudinal changes in cytokine level during ART and intact HIV kinetics. For each 10-fold increase of MIP-3a over time, we observed a concurrent 9.5% faster decay of intact HIV genomes ($p = 0.021$), while for each 10-fold reduction of IL-6, intact genomes decreased 10% faster per year ($p = 0.043$) (Figure 1b).

Conclusions: The extent of intact HIV decay was predicted by baseline galectin-9 levels, while MIP-3a and IL-6 correlated with intact HIV kinetics. Galectin-9 was the host factor most strongly associated with subsequent intact HIV decay, in alignment with its established roles in regulation of HIV expression and cytotoxic immunity.

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OP 4.8 – 00149

CD8+ T cells promote hiv latency in CD4+ T cells through the downmodulation of NF-kB

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Background: The main obstacle to achieving a cure for HIV infection is the presence of cells harboring the HIV reservoir, despite antiretroviral therapy (ART). Previous studies have shown that CD8+ T cells (CD8+) promote HIV latency, however, this mechanism is poorly understood. Here we show that non-cytolytic CD8+ negatively regulate HIV expression in the memory CD4+ T cell (mCD4+) subsets by downmodulating the activity of the HIV transcriptional regulator NF-kB.

Methods: We modified our in vitro model of HIV latency to study the mechanism of HIV suppression exerted by CD8+. Both resting and TCR-activated mCD4+ were HIV-infected in vitro and cocultured with autologous activated CD8+ in the presence of ART for three days. mCD4+ monocultures were used as control. After co-culture, mCD4+ and CD8+ were FACS-sorted and mCD4+ were returned to culture for 72 hours. The mCD4+ subset distribution and HIV expression were monitored by flow cytometry. Infection frequency was quantified by integrated HIV DNA qPCR. Modulation of NF-kB targets was analyzed by RT-PCR at 24, 48 and 72 hours post co-culture.

Results: Following co-culture with CD8+, effector, transitional and central mCD4+ subsets showed a significant reduction of HIV-Gag expression, without impacting the memory subset distribution. To determine the effect of CD8+:CD4+ co-culture on the HIV transcriptional regulator NF-kB, we measured the expression of the NF-kB targets IL-6, IFN-g, and NF-kB-p65. We found that co-culture with CD8+ induced a significant reduction in NF-kB targets in resting and activated mCD4+. The reduction in Gag expression and NF-kB activity were sustained for up to 72 hours after isolation of mCD4+ from the CD8+ co-cultures.

Conclusions: Our results demonstrate that CD8+ promote the establishment of HIV latency by downmodulating HIV expression. CD8+ downmodulate NF-kB in mCD4+ independently from HIV infection, suggesting that their pro-latency activity might be due to the NF-kB suppression. After CD8+ removal from coculture, NF-kB activity and Gag expression do not return to levels comparable to monoculture, highlighting the existence of a sustained pro-latency effect exerted by CD8+. Understanding the mechanism of NF-kB modulation in mCD4+ may represent a HIV latency reversal tool to develop a new cure strategy.

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PP 4.1 – 00013

Vaccine-mediated induction of elite control-associated CD8+ cytotoxic T lymphocytes in Mamu-B*08+ Indian rhesus macaques does not protect against intrarectal SIVmac239 acquisition

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Background: Indian rhesus macaques (RMs) expressing the *Mamu-B*08+* MHC I allele exhibit a predisposition to controlling chronic-phase SIVmac239 viremia. Indeed, ~50% become elite controllers (ECs) after infection, with chronic-phase viremia below 1,000 vRNA copies/mL. Elite control appears to be mediated by Mamu-B*08-restricted CD8+ cytotoxic T lymphocytes (CTLs) that recognize immunodominant Vif- and Nef-derived epitopes. While prophylactic vaccination with *vif* and *nef* increases EC incidence, it is unclear whether these vaccine-induced CTLs can provide protection against SIVmac239 acquisition.

Methods: Sixteen *Mamu-B*08+* RMs were vaccinated with Ad5, VSV, and rhesus rhadinovirus (RRV) vectors encoding SIVmac239 Vif and Nef in a heterologous prime-boost-boost regimen. Vaccine immunogenicity was evaluated by pMHCI tetramer staining and flow cytometric analysis of PBMCs at timepoints two weeks after each vaccine dose. The 16 vaccinees and 16 unvaccinated *Mamu-B*08+* controls were subjected to biweekly intrarectal challenges with a marginal infectious dose of SIVmac239. Plasma viral loads were measured at day 7 and day 10 timepoints following each challenge to confirm infection status.

Results: All 16 vaccinees mounted CTL responses against the Vif RL8 and Nef RL10 epitopes. of Nef RL10-specific CTLs, 45.8% were granzyme B+ and 53.3% were TEM2 (CD28– CCR7–), on average, two weeks after the final RRV boost. Collectively, the four immunodominant Mamu-B*08-restricted CTL populations accounted for an average of 1.11% of circulating CD8+ T cells following the RRV boost. The rate of SIVmac239 acquisition did not differ between vaccinees and unvaccinated controls ($P = 0.2989$ by log-rank/Mantel-Cox test). However, peak viremia was significantly lower in vaccinees than in controls ($P < 0.0001$ by Mann-Whitney U test), and geometric mean viral loads were generally at least tenfold lower in vaccinees than in controls throughout the course of infection.

Conclusions: Our vaccination regimen elicited SIVmac239 elite control-associated CTL populations in *Mamu-B*08+* RMs. Vaccination did not provide significant protection against intrarectal SIVmac239 acquisition but did appear to facilitate reductions in peak and setpoint viremia. We conclude that boosting elite control-associated CTLs by vaccination is inefficacious in providing sterilizing immunity, but that it does appear to reduce disease severity and slow progression to AIDS once an animal is infected.

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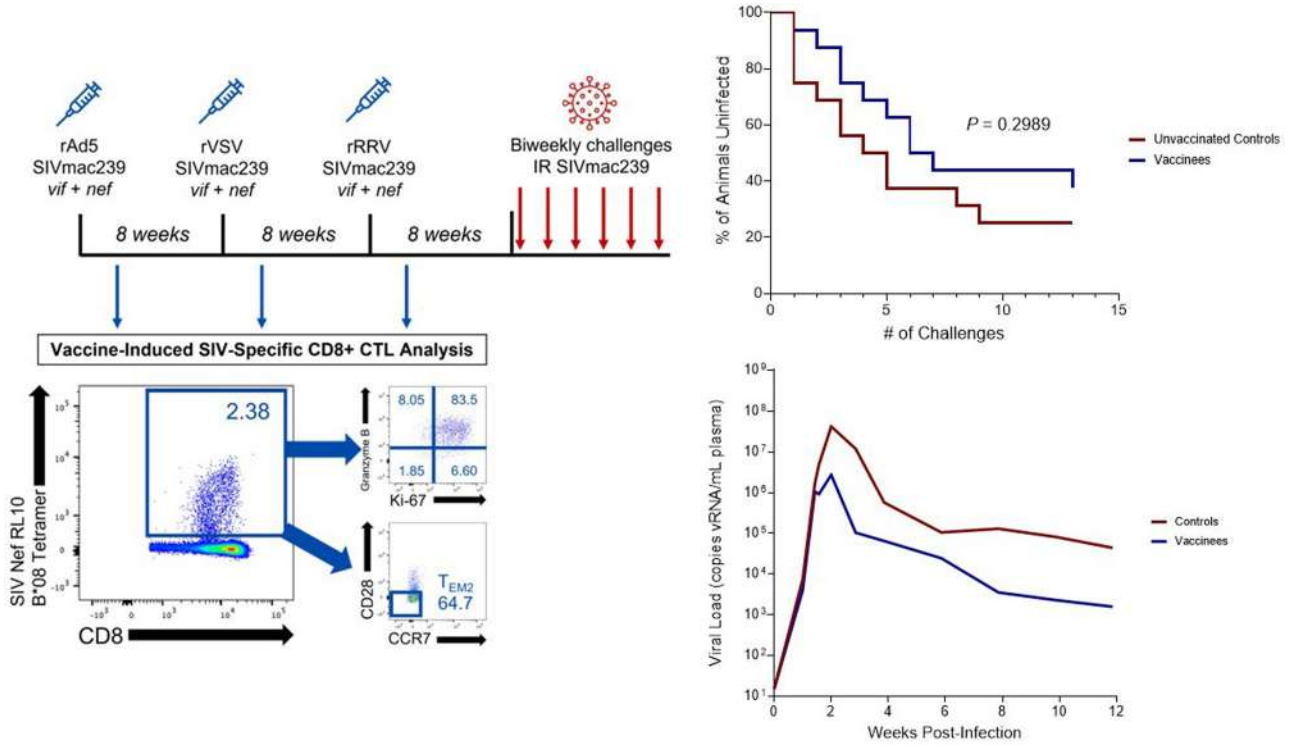

Figure 1 (abstract: PP 4.1–00013) B*08+ Vaccination and SIVmac239 Challenge Study

P 4.2 – 00029

Innate Immune Correlates of Cell-Associated HIV RNA And DNA During Long-Term Suppressive ART

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Background: Persistence and clonal expansion of HIV-infected cells during long-term antiretroviral therapy (ART) prevents HIV cure in persons living with HIV (PLWH). Replication-competent and -incompetent proviruses persist during ART, and both may contribute to HIV pathogenesis, but mechanisms driving their persistence remain unclear. To investigate the potential role of immune-activation in persistence of proviral populations, we analyzed cell-associated HIV RNA and DNA levels in the context of cellular immunophenotyping.

Methods: Clinical information and peripheral blood mononuclear cells (PBMC) were obtained from PLWH in IRB-approved protocols undergoing ART for ≥3 years (y). Levels of cell-associated HIV LTR and *gag* RNA and DNA were measured by multiplexed droplet digital PCR. PBMC were analyzed in flow cytometry panels quantifying >20 lymphocyte subsets and activation markers. We analyzed clinical, immunophenotyping and virologic data with parametric and nonparametric methods to detect significant correlates and identify composite sets of parameters with highest Pearson correlation by multilinear regression.

Results: Participants (N = 73, 11% female, average age 49.9 [range 20–70] y, median CD4 = 639 [range 250–1765] cells/μl at sampling) had undergone continuous ART for median 14 (range 3–29) y. Levels of HIV LTR DNA (median 2500 copies/million CD4 cells) varied over 100-fold (59.9–52508 copies/million CD4 cells) and were highly deleted in *gag* (average LTR:gag DNA ratio 5.1, range 0.7–48.6). Regression analyses revealed levels of proviruses were strongly associated with proportion of CD8+ memory cells, duration of infection, and nadir CD4+ count (for the composite, Pearson $r = 0.57$, $p < 0.001$). HIV RNA data was available from 58/73; levels of cell-associated LTR RNA varied >100-fold (0.02–4.3 copies HIV LTR RNA/provirus). Analyses of HIV *gag* RNA revealed even lower levels (0.002–2.4 *gag* RNA copies/gag-containing proviruses). In best fit analyses, levels of LTR RNA/provirus were highly correlated in models composed of %CD15+CD56+ natural killer (NK) cells in PBMC and age (Pearson $r = 0.59$, $p < 0.01$).

Conclusions: After long-term ART, HIV proviruses are highly deleted. Levels of cell associated HIV LTR RNA were strongly associated with proportion of NK cells in peripheral blood, suggesting innate immune sensing of HIV RNA.

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PP 4.3 – 00052

Intra- and extracellular levels of acyl-coA-binding protein and anti-HIV T-cell function in people living with HIV

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Background: Autophagy, a lysosomal degradation pathway catabolizing cytosolic structures to produce energy, allows the establishment and maintenance of efficient anti-HIV T-cell responses. Intracellular acyl-CoA-binding protein (ACBP) favors autophagy by shuttling activated fatty acids to cellular organelles. Conversely, autophagy is inhibited when ACBP is secreted into the extracellular space. As autophagy is associated to efficient T-cell function, we assessed intra- and extracellular ACBP levels in people living with HIV (PLWH) under antiretroviral therapy (ART). As autophagy-dependent glutaminolysis is critical for IL21 production for optimal HIV-specific T-cell responses, metabolomic analyses were performed to assess involvement of the tricarboxylic acid cycle metabolites.

Methods: Plasma ACBP and cytokine levels were assessed by ELISA in 60 long-term (median 14.7 years) ART-treated PLWH and 30 uninfected controls. Intracellular ACBP levels were assessed by flow cytometry in PBMC. Metabolomic analyses were performed on serum samples by LC-MS.

Results: Plasma ACBP levels were higher in ART-treated PLWH compared to controls (medians 127.5 vs 78.1 ng/mL, $p = 0.03$), independently of age, sex and weight. Intracellular ACBP was detected in all leukocytes in both groups. Plasma ACBP levels correlated negatively with its intracellular levels in T-cells ($r = -0.9$, $p = 0.02$) and monocytes ($r = -0.9$, $p = 0.08$) suggesting that low plasma levels of ACBP are associated with cellular retention of the protein.

In ART-treated PLWH, plasma ACBP levels were neither associated with CD4, CD8 T-cell counts nor GDF15 nor FGF21 levels, but correlated with levels of growth factors (EGF, G-CSF, GRO), pro-inflammatory cytokines (IFNα2, IFNγ, IL1β) and homeostatic factors (IL7 and IL15) ($r > 0.3$, $p < 0.05$ for all). Plasma ACBP levels were inversely associated with plasma IL21 levels ($r = -0.54$, $p < 0.01$). PLWH with plasma ACBP levels above the median had two-fold higher levels of glutamic acid ($p = 0.02$), a higher glutamic acid/glutamine ratio ($p = 0.03$) and tended to have higher levels of α-ketoglutarate (1.5-fold difference, $p = 0.09$) in their serum.

Conclusions: Higher plasma levels of ACBP in ART-treated PLWH were associated with inflammation, oxidative stress, and markers of T-cell dysfunction. Our findings indicate that circulating ACBP might weaken anti-HIV T-cell functions in an IL21-dependent manner. The ACBP pathway might constitute a target for improving anti-HIV T-cell responses.

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PP 4.4 – 00059

Quantification of HIV Reservoirs in Brain: focus in bystander damage

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Background: Early after primary infection, HIV reservoirs are established within multiple tissues, including the brain. As these viral reservoirs are not targeted by antiretroviral therapy (cART), we require robust methods of detection, quantification, and characterization of these viral reservoirs in human tissues. Our recent work developed a multi-component imaging methodology that characterizes and quantifies viral reservoirs within the brain.

Methods: The imaging methodology demonstrated utilizes the simultaneous staining of brain tissue from HIV-infected donors using DNAscope, RNAscope, and antibodies for HIV-DNA, HIV-mRNA, and either viral or host proteins, respectively. The panel of patients included in these analyses varied in cART regimen, viral load, years living with HIV, and neurocognitive status, all contrasted to age-matched tissues from uninfected patients.

Results: Our group demonstrated that cART is sufficient to reduce the size of the viral reservoirs within the brains of HIV patients. We also found that about half of the cells positive for HIV-DNA expressed HIV-mRNA, and only about one-third expressed viral proteins. HIV proteins varied in expression and bystander uptake by uninfected cells but could provide insight into bystander toxicity.

Conclusions: The results found were present irrespective of cART regimen and systemic viral replication but suggested that these viral reservoirs are a major barrier to curing HIV and treating associated neurocognitive disorders.

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PP 4.5 – 00064

Differentiation Enhances Reactivation of Latent HIV-1 Reservoir in CD4+ T Cells in PLWH With > 4 Years of Viral Suppression

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Background: Accurately quantifying the latent HIV-1 reservoir in resting CD4+ T cells (rCD4) in people living with HIV (PLWH) on antiretroviral therapy (ART) remains a significant barrier to curative approaches. Molecular measurements cannot discriminate replication fitness of intact provirus, while the Quantitative Viral Outgrowth Assay (QVOA) has been shown to underestimate the viral reservoir frequency. We previously reported that *ex vivo* differentiation of rCD4 to an effector memory phenotype in viral outgrowth assays significantly enhanced HIV-1 latency reversal and demonstrated the replication competent reservoir size was on average 18-fold higher than had been estimated. Here, we used the differentiation QVOA (dQVOA) to further refine the relationship between rCD4 differentiation, persistence of the reservoir, and time on ART.

Methods: rCD4 were isolated from cryopreserved PBMCs from 32 ART-suppressed PLWH with short, intermediate, and long-term ART suppression (1.8 ± 0.8, 4.1 ± 0.9, and 12.1 ± 3.1 years, respectively). We assessed the frequency of cell-associated HIV RNA by real-time PCR, ca-total and integrated HIV DNA by real-time PCR, and infectious units per million (IUPM) CD4+ T cells via both QVOA and dQVOA in parallel. *Ex vivo* and resting-enriched CD4+ T cell phenotypes were assessed by flow cytometry.

Results: Results from standard QVOA showed long-term ART suppression was consistent with a decay in the reservoir frequency. By contrast, dQVOA run in parallel from the same pool of rCD4 enriched from each participant revealed significantly higher IUPM values from individuals with intermediate ($p = 0.0056$) or long ($p < 0.0001$) term ART suppression. Molecular measurements supported no significant decrease in reservoir with long-term ART. Analysis of the effector memory frequency in the *ex vivo*, resting-enriched, and post-differentiation CD4+ T cell subsets revealed a significant positive correlation with dQVOA IUPM, whereas no such relationship was observed in standard QVOA. This observation suggests long-term ART may not result in a smaller reservoir size, but instead, CD4+ T cells from long-term suppressed PLWH may require additional signaling for effective viral reactivation.

Conclusions: Together, these data suggest the replication competent reservoir is stable over time, and more targeted approaches are required to reactivate latent HIV after long-term viral suppression, providing crucial insights into designing and evaluating HIV curative approaches.

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PP 4.6 – 00065

Retinoic Acid Transcriptionally Reprograms Macrophages for Increased Permissiveness to HIV-1 Replication

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Background: The intestinal environment facilitates HIV-1 replication/viral reservoir (VR) persistence via mechanisms involving the gut-homing elixir retinoic acid (RA), which transcriptionally reprograms CD4+ T-cells for increased HIV-1 infection. Consistently, colon-infiltrating CD4+ T-cells carry viral reservoirs (VR) in people living with HIV-1 (PLWH) receiving antiretroviral therapy (ART). Intriguingly, integrative infection in colon macrophages, a pool constantly replenished by circulating monocytes, represents a rare event in ART-treated PLWH, thus raising questions on HIV-1 permissiveness in gut-residing macrophages. Indeed, the persistence of VR in long-lived CD4+ T-cells of people living with HIV (PLWH) receiving viral-suppressive antiretroviral therapy (ART) is well-established, while the contribution of macrophages (MΦ) remains a subject of debate. We hypothesized that, in an environment rich in RA, MΦ are highly permissive to HIV-1 replication and contribute to the initial viral transmission and viral rebound upon ART interruption. Thus, we aimed (i) to explore the effect of RA on HIV-1 replication and (ii) to identify molecular mechanisms by which RA modulates HIV-1 replication in MDM in vitro.

Methods: Monocyte-derived MΦ (MDM) were generated in the presence/absence of RA in vitro. CCR5 and CXCR4 expression was measured by FACS. MDM were infected with replication-competent CCR5-tropic and VSV-G-pseudotyped HIV molecular clones. HIV replication was measured by ELISA, FACS, and PCR using specific primers for early/late reverse transcription and integrated HIV-DNA. RNA-Sequencing was performed to identify RA-mediated HIV permissiveness factors in MDM. Validations for mTOR, S6K, TCF4, HIC1, IL-10 and PPARg were performed.

Results: Here, we demonstrate that RA promotes HIV-1 replication in MDM via entry/post-entry mechanisms. RNA-Sequencing, together with Gene set variation analysis and HIV interactor NCBI database interrogation revealed profound RA-mediated transcriptional reprogramming associated with effector functions, metabolic/signaling status, and HIV-1 dependency/restriction factors. Functional validations and pharmacological antagonism demonstrated the contribution of RA-modulated mTOR/S6K and Wnt/b-catenin/TCF4 pathways.

Conclusions: These results support a model in which macrophages in RA-rich tissues represent important HIV-1 targets contributing to viral dissemination/replication before/upon ART interruption. Therefore, the rarity of viral reservoir in colon-infiltrating MDM of ART-treated PLWH is likely not the consequence of their resistance to infection but rather due to their rapid turn-over in vivo.

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PP 4.7 – 00097

Signatures of HIV-Infected CD4+ T Cell Resistance to NK Cell-Mediated Cytotoxicity

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Background: HIV-infected CD4+ T cells persist ex vivo despite treatment with a latency reversal agent (LRA) in combination with CD8+ T cells, known as the "Shock-and-Kill" approach. Similarly, in vitro experiments in which HIV-infected CD4+ T cells are co-cultured with natural killer (NK) cells yield incomplete elimination of the infected cells. We therefore hypothesize that cell-intrinsic pathways in CD4+ T cells are differentially regulated in HIV-infected cells to mediate resistance to NK cell-mediated killing.

Methods: Mock-infected and HIV-89.6-infected CD4+ T cells from 6 donors were co-cultured overnight +/- autologous NK cells. Following co-culture, cells were stained for the surface exposed HIV envelope protein using fluorescently conjugated HIV antibodies. Fluorescence activated cell sorting (FACS) was then used to isolate infected and uninfected cells, followed by bulk RNA-sequencing. Transcripts from infected and uninfected cells co-cultured overnight with versus without NK cells were compared.

Results: RNA-seq analysis of CD4+ T cells that survived co-culture with NK cells revealed dozens of differentially expressed genes in infected cells, but not uninfected cells. This included upregulation of multiple interferon-stimulated genes, including PD-L1 ($p = 0.0086$), and genes involved in NF-kB signaling. Additional upregulated genes in the surviving infected cells included c-Fos ($p = 0.017$), c-Jun ($p = 0.004$), and cathepsin L ($p = .0112$).

Conclusions: This dataset will be used to guide future experiments to uncover which genes actively contribute towards resistance to killing versus genes that are regulated as a consequence of survival. For example, NF-kB genes that are downregulated in infected cells, potentially through Vpu activity, are normalized in survived infected cells. This may be due to NK cell targeting of Vpu-mediated HLA-C-downregulation. In contrast, the induction of IFN-stimulated genes may result from exposure of survived cells to NK cell-derived cytokines. As RNA-seq will not capture post-translational modifications of NK cell ligands, such as MHC-I downregulation mediated by HIV accessory proteins, follow up studies include CITE-Seq to

determine the differences in surface protein expression of infected cells that survive interactions with NK cells. Deconvolution of relative contributions of specific genes/proteins to target cell resistance will reveal novel targets for the development of therapeutics to eliminate the HIV reservoir.

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PP 4.8 – 00108

Presentation of cognate antigens by dendritic cells causes stochastic HIV expression

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Background: Encounter with cognate antigens (Ag) is a major cause of proliferation and persistence of HIV-infected CD4+ T cells. Understanding to which extent physiological T cell activation elicits latency reversal is key for cure efforts. Owing to the low frequency of HIV-infected Ag-reactive cells, previous studies failed to compare HIV reactivation mediated by Ag recognition versus global T cell activators.

Methods: CD8-depleted PBMCs from 10 PLWH on ART were stimulated with either CMV or HIV Gag antigens. Reactive cells (CD40L+) were isolated and expanded for 10–14 days. Expanded pools were characterized by TCR repertoire, total and intact HIV DNA, and proviral sequencing. After resting for 3 days, cells were re-stimulated with either PMA/Io, anti-CD3/CD28, or autologous dendritic cells (DCs) pulsed with cognate or unrelated Ag (KLH). We measured T cell activation by CD40L and CD69 expression, and profiled HIV RNA by bulk and limiting dilution digital PCR and sequencing.

Results: Our approach allowed us to isolate rare Ag-reactive CD4+ T cells (range 0.5–2%) and expand them while preserving their overall TCR repertoire. The frequency and composition of proviruses in the expanded pools were highly variable across participants. While most HIV genomes were defective, we detected intact proviruses in 2/5 and 5/5 participants' CMV or Gag reactive cells, respectively. Upon restimulation, only cognate Ag caused significant T cell activation (CMV 69.57%, Gag 64.4%, KLH 4.08%, $p < 0.0001$). Cognate Ag induced increased HIV expression, but this increase showed inter-participant variability (fold change to baseline 0.83–50.56, $p = 0.004$). While for 2 participants we observed high HIV expression (48- and 50-fold), 4/10 participants exhibited little HIV RNA increase (fold change to baseline 0.8–4). Conversely, treatment with PMA/Io and anti-CD3/CD28 increased HIV RNA production across all participants (fold change mean 13.5 and 6.5, respectively). Limiting dilution RNA assays showed similar breadth of proviruses induced across conditions, but only PMA/Io stimulation caused high HIV RNA-producing cells (>102 cp/cell).

Conclusions: These results imply quantitative and qualitative differences in cellular and HIV transcriptional profiles when CD4+ T cells encounter their cognate Ag compared to strong T cell activators. Our work suggests that, for some proviruses, physiological T cell activation is insufficient for latency reversal.

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PP 4.9 – 00113

Transcription of Defective HIV Proviruses Trigger Innate Immune Responses

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Background: HIV persistence is driven by a subset of cells harboring transcriptionally repressed latent HIV that contributes to rebound upon treatment interruption presenting a challenge to HIV cure. However, examination of the persistent viral sequences has shown that the reservoir consists of mostly defective viruses with point mutations, frame shifts, inversions, and deletions which would limit productive HIV-1 transcription. Furthermore, even in the presence of antiretroviral therapy and limited HIV transcription, people living with HIV (PLWH) demonstrate

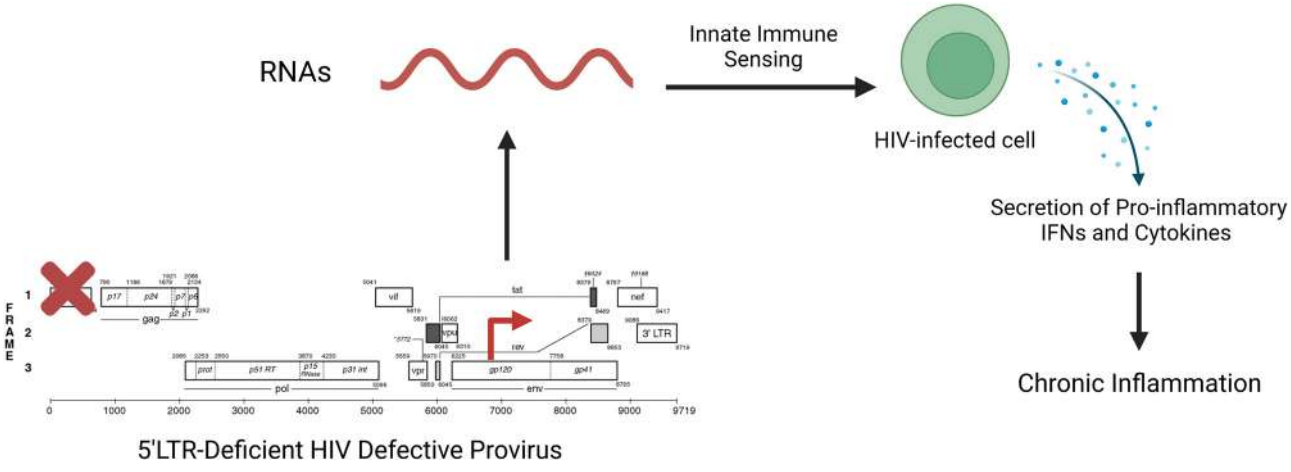

Figure 1 (abstract: PP 4.9–00113) Innate Immune Response to Defective HIV Proviruses

chronic comorbidities of the central nervous system, heart, and general inflammaging. It is unknown what drives the dysregulated inflammation in PLWH. We hypothesize that the expression of defective proviruses activates innate immune activities in T cells and myeloid cells to perpetuate inflammation. This hypothesis is supported by previous work where we demonstrated expression of RNAs and proteins from defective proviruses driven by an intragenic promoter.

Methods: We have employed CRISPR-cas9 to engineer cells harboring defective HIV proviral genomes lacking 5′LTRs. Expression of Type I IFN-stimulated genes was measured using qPCR, while expression of HIV transcripts was measured using ddPCR.

Results: Induction of IFN-stimulated genes, including IP10, ISG15, and MX1, was significantly higher in cells harboring defective proviruses than those harboring mostly intact virus. This expression correlated with the presence of the intragenic promoter and higher levels of defective HIV transcripts, suggesting that these transcripts elicit an innate immune response.

Conclusions: We propose a model whereby defective proviruses produce RNAs which are detected by innate immune sensors, leading to activation of pro-inflammatory ISGs and cytokines. These processes contribute to chronic inflammation observed during persistent infection in PLWH on suppressive ART. Further studies will elucidate the mechanisms involved in this process and improve understanding of HIV-1 mediated immune dysfunction and pathogenesis.

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PP 4.10 – 00119

Multiomic dynamics of the cellular HIV reservoir after rebound during ATI

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Background: The complex pool of infected cells that comprise the HIV reservoir can be distributed amongst CD4+ T subsets with varied functional and compartmental characteristics. Recent studies during treatment interruption in passive immunotherapy trials have demonstrated that reservoir reseeding can coincide with viral rebound. However, whether reseeding is associated with compositionally distinct cellular populations is unknown.

Methods: We profiled the reservoir from three participants of the clinical trial (ACTG A5340) who experienced viral rebound after receiving the broadly neutralizing antibody VRC01 during analytical treatment interruption (ATI). Pre-ATI and post-ATI blood samples were collected while viral load was fully suppressed. We applied viral single-cell Assay for Transposase Accessible Chromatin with Select Antigen Profiling by sequencing (ASAPseq) to identify HIV+ cells using accessible proviral DNA and their coordinate cell surface markers. Peripheral blood memory CD4+ T cells were enriched by bead separation and labeled with oligo-tagged antibodies for generation of viral ASAPseq libraries. Reads were processed using our custom pipeline which included alignments to consensus and autologous viral sequences.

Results: We profiled 136997 memory CD4+ T cells with viral ASAPseq, of which 205 cells (0.15%) were detected as HIV+. After clustering and annotating with epigenetic and surface antigen data, we compared the phenotypes between the pre-ATI and post-ATI timepoints for each individual. In one individual with a low viral rebound (as determined by area under the curve; AUC) during ATI, phenotypic composition of HIV+ cells was maintained. In contrast, the other individuals with higher viral load rebound had greater disruption of the phenotypic composition of HIV+ cells. Reservoir modulation was specifically associated with the emergence of recently activated Tcm/Ttm cells at the post-ATI timepoint.

Conclusions: Our observations suggest that the extent of viral rebound AUC is associated with greater changes in reservoir phenotype, whereupon incomplete viral suppression during clinical trial interventions can lead to diversification of the cellular phenotypes found in the HIV reservoir. Furthermore, these findings highlight the necessary use of single-cell based methodologies to understand phenotypic perturbations of the heterogenous HIV reservoir.

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PP 4.11 – 00126

Autologous Neutralizing Antibodies Increase with Early Antiretroviral Therapy and Shape HIV Rebound after Treatment Interruption

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Background: Early initiation of antiretroviral therapy (ART) alters viral rebound kinetics after analytic treatment interruption (ATI) and may play a role in promoting HIV remission in people with HIV (PWH). Autologous neutralizing antibodies (aNAbs) represent a key adaptive immune response in PWH. We aimed to investigate the role of aNAbs in shaping post-ATI HIV rebound variants.

Methods: We performed single-genome amplification of HIV-1 env from pre-ART and post-ATI plasma samples of 12 early treated individuals. aNAb activity was quantified using pseudoviruses derived from the most common plasma variant and the serum dilution that inhibited 50% of viral infections (ID50) was determined.

Results: aNAb responses matured significantly while on suppressive ART as early post-ATI plasma demonstrated significantly improved neutralizing activity against pre-ART HIV strains compared to pre-ART plasma (median ID50 titer [1/x]: 19 vs. 353, $P = 0.002$). Post-ATI aNAb responses exerted selective pressure on the rebounding viruses as the post-ATI HIV strains were significantly more resistant to post-ATI plasma neutralization compared to the pre-ART virus (median 353 vs. 27, $P = 0.04$). Several pre-ATI features distinguished post-treatment controllers (PTCs) from non-controllers (NCs), including infecting sequence more similar to consensus B, more restricted proviral diversity and a stronger aNAb response. Post-treatment

control was also associated with the evolution of distinct N-glycosylation profiles in the HIV Env.

Conclusions: Autologous neutralizing antibody responses mature after early initiation of ART and applies selective pressure on rebounding viruses. The combination of aNAb activity with select HIV sequence and reservoir features identified individuals with a greater chance of post-treatment control.

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PP 4.12 – 00141

Enhancement of IL15/IL15RA signaling in immune cells using CRISPR-dCas9-VPR platform

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Background: NK (natural killer) and CTLs (cytotoxic T cells) functions are positively regulated by IL-15 signaling (Fig 1A). The IL-15 receptor comprises three subunits: IL15R alpha, beta, and gamma. The IL-15/IL-15RA dimer can be trans-presented by myeloid cells to CD4+ and CD8+ cells and NK cells, which express the beta and gamma subunits. In addition, CD8+ T cells and NK cells can also express IL15RA upon stimulation and trigger IL15 signaling through cis-presentation. Specific upregulation of IL15/IL15RA expression/signaling in CTLs should improve their proliferation and cytolytic functions and thus lead to better clearance of HIV-infected cells. CRISPR-Cas9 platforms using catalytically dead Cas9 (dCas9) protein allow precise control of genome expression without gene editing. Here to accomplish potent transactivation of the transcription of IL15RA and IL15 in immune cells, we utilized the VPR platform where dCas9 is fused to linked in tandem VP64-p65-Rts transcription activation domains.

Methods: Based on a literature search, we identified NFkB motifs and CpG islands in the promoter regions of the target genes IL15RA and IL15 (Fig 1B,C). Next, the promoter region sequences of these genes were analyzed for gRNAs binding sites, and 18 (IL15RA, Fig 1D) or 14 (IL15, Fig 1E) gRNAs were selected for further testing. K562 lymphoblast cell line stably expressing dCas9-VPR was electroporated with synthetic gRNAs, including non-targeting gRNA as a control. After three days, the mRNA expression of target genes was examined by qRT-PCRs. In addition, the IL15RA expression was checked by immunolabeling/flow cytometry and IL15 release from treated cells by ELISA. Additionally, cell proliferation and viability were measured by PI staining followed by flow cytometry.

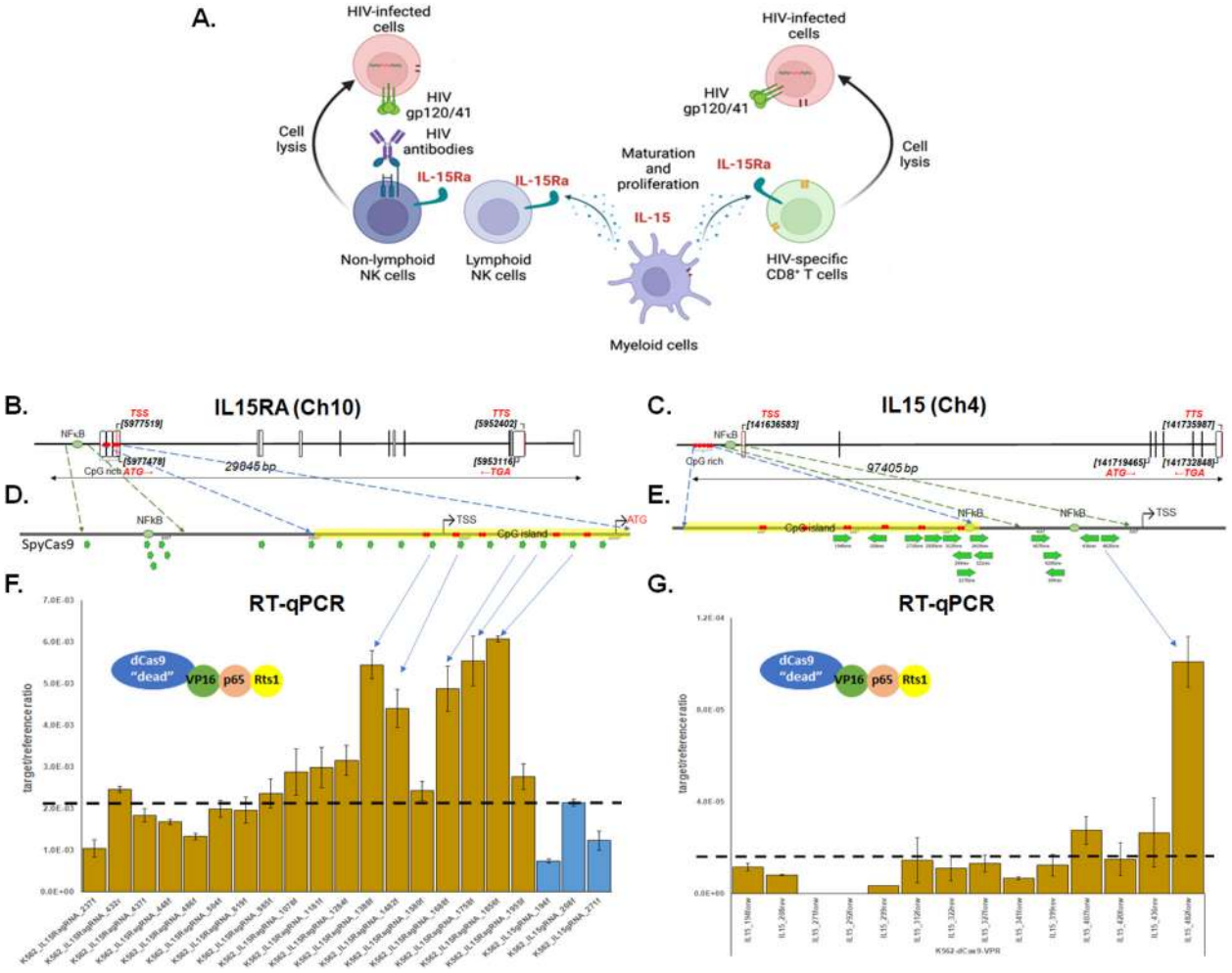

Figure 1 (abstract: PP 4.12–00141)

Results: We identified regions of IL15RA and IL15 promoters responding to dCas9-VPR-mediated transcription transactivation. gRNAs recruiting dCas9-VPR into the vicinity of transcription start sites showed the most potent induction of the target genes transcription (Fig 1FG). The effects of dCas9-VPR were not dependent on the presence of NFkB enhancer regions and CpG islands.

Conclusions: The CRISPR-dCas9-VPR platform can be used for specific and efficient enhancement of IL15/IL15RA expression in target immune cells.

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PP 4.13 – 00151

Soluble Factors Drive Naïve CD4+ T Cells to Differentiate into CCR5 + Tissue Resident Memory Cells that are Highly Susceptible to HIV infection

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Background: Persistent HIV reservoirs are established in the gut within the first weeks of infection. These infected cells are a barrier to an HIV cure. Tissue resident $CD4^+$ memory T cells (T_{RM}s) reside in gut tissues and may be involved in the formation of viral reservoirs. MAdCAM, which is expressed on gut endothelium delivers costimulatory signals to $CD4^+$ T cell and supports HIV replication. We have proposed that this type of costimulation could help explain how gut tissues are rapidly and preferentially infected in the acute stage of HIV infection. However, the manner in which $CD4^+$ T cells differentiate following MAdCAM costimulation is not known.

Methods: To understand how cells stimulated with MAdCAM differentiate, we carried out gene expression profiling by RNAseq following MAdCAM costimulation of primary $CD4^+$ T cells. We also measured cytokine production using multiplex cytokine assays. On stimulated CD4+ T cells was also performed a multicolor flow-cytometric analysis.

Results: We report that MAdCAM and retinoic acid (RA), two constituents of gut tissues, together with TGF-β, promote the differentiation of CD4+ T cells into a distinct subset α4β7+ CD69+CD103+ T_{RM}s. Among the costimulatory ligands we evaluated, MAdCAM was unique in its capacity to upregulate high levels of both CCR5 and CCR9. These cells are susceptible to HIV infection, despite the anti-proliferative action of TGF-β. Formation of TRMs was reduced by MAdCAM antagonists developed to treat inflammatory bowel diseases.

Conclusions: These finding provide a framework to better understand the contribution of CD4+ TRMs to persistent viral reservoirs and HIV pathogenesis.

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PP 4.14 – 00173

Antiretroviral therapy repairs CD4 T cell dysregulation in people living with HIV

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Background: Recent studies show that the majority of the HIV reservoir is stabilized at the time of ART initiation. We hypothesized that ART in people with HIV (PWH) contributes to this stabilization by restoring CD4 T cell memory formation.

Methods: We used a 32-marker mass cytometry panel to examine CD4 T cell memory dynamics in PWH durably (~5yrs) suppressed with ART (PWHART, n = 10) and PWH in the 1.5 years following ART initiation (n = 10, ACTG5248). The panel included markers of activation (HLA-DR, CD38, CCR5), activation/exhaustion (PD-1), proliferation (Ki67), survival (Bcl-2) and long-lived memory (CD127). We paired these CD4 T cell studies with functional analysis of proliferation and T cell receptor (TCR) β clonotype sequencing.

Results: Using fixed effects models with indicators for participant ID, we found that frequencies of CD4 T cell memory subsets and expression of activation, exhaustion, cell cycling and long-liveness are remarkably stable in PWH-ART. In contrast, after ART initiation, activation and cell cycling decreased within weeks whereas markers of T cell long-liveness, CD127 and Bcl-2 increased slowly over months. Expanded CD4 T cell receptor (TCR) clonotypes (3.3–10.8%) were detected at ART initiation and were maintained at these frequencies for the first 18 months of ART suppression. CD4 TCR clonotypes were also maintained in PWHART but relative frequencies were lower (0.3–6.8%). To assess functionality of CD4 T cells post-ART, we measured CD4 T cell proliferation in response to pp65/IE1 HCMV and mitogen stimulation. HCMV-specific and mitogen-reactive proliferation was clearly detectable at ART initiation and decreased as viral load was suppressed but increased 3 months post ART initiation. By contrast, we did not detect HIV Gag/Nef-specific CD4 T cell proliferation over background at any timepoints measured.

Conclusions: In our study population (CD4 nadir 77–453), CD4 T cells were highly activated but retained proliferative capacity at ART initiation. ART resulted in rapid decreases in cell cycling and activation. Interestingly, restoration of CD4 T cell memory phenotypes was much slower, occurring over the months-years following virus suppression. Overall, our data suggest that immune changes at ART initiation help stabilize the HIV reservoir in CD4 T cells.

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PP 4.15 – 00181

Distinctive cytoskeletal properties are implicated in the resistance of a fraction of productively HIV-infected CD4 + T-cells to killing by cytotoxic T lymphocytes (CTL)

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Background: Although latency is a primary enabler of HIV persistence, recent studies have highlighted the existence of infected-cell clones that express HIV on ART. Evidence of ongoing antigenic stimulation of HIV-specific CTL, and gradual decreases in intact and transcriptionally active proviruses over years on ART suggest some ongoing CTL pressure. We hypothesized that the selection of survivors with CTL-resistant properties may limit this process. Here we directly investigate the existence and underlying mechanisms of CTL resistance in productively HIV-infected CD4 + T-cells.

Methods: Central memory CD4 + T-cells (Tcm) from n = 3 HLA-B58 + and HLA-A02 + donors were infected separately with one of two HIVJRSCF variants: differing by the presence/absence of an escape mutation in the Gag-TW10 CTL epitope. These were dyed with CTFR (WT) or CFSE (TW10esc), mixed, and co-cultured with TW10-specific CTL clones. Whether CTFR 'survivors' were resistant relative to CFSE 'bystanders' were assessed by flow cytometry following a second co-culture with a CTL clone targeting an epitope present in both viruses (Gag-SL9). Bystanders and survivors (sorted on HIV-Env + cells) were profiled by RNA-sequencing.

Results: 90% of cells infected with WT-HIVJRSCF were eliminated by TW10-specific CTL, and the survivors remained CTL resistant in the second round of exposure to SL9-specific CTL ($p = 0.98$). In contrast, cells infected with TW10esc-HIVJRCSF, which were bystanders in first-round killing, were efficiently eliminated by SL9-specific CTL ($p = 0.01$). In the absence of CTL, WT and TW10esc infected Tcm had very similar transcriptional profiles (35 differentially expressed genes (DEGs), padj < 0.05). In striking contrast, WT Tcm that survived CTL had distinctive transcriptional profiles from TW10esc bystanders (2,234DEGs, padj < 0.05). Amongst the biological processes implicated by gene set enrichment analysis were deficiencies in cytoskeletal regulation and cell-cell junction in survivors (NES-1.59, adj.p < 0.003; NES-2.04, adj.p < 7.31e-05). Preliminary assessments by acoustic scattering suggest that surviving cells are more deformable when compared to bystanders ($p = 1.6e-08$).

Conclusions: Amongst HIV-expressing cells, there is heterogeneity in intrinsic susceptibility to CTL- with resistant cells exhibiting distinctive transcriptional profiles. Initial results implicate a role in cytoskeletal regulation and mechanical stiffness. These mirror observations from immuno-oncology– where cell-stiffing agents have improved CTL-tumor synapse formation and pre-clinical outcomes. Such approaches warrant testing to improve the elimination of HIV-infected cells.

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PP 4.16 – 00188

Investigating the Role of Naïve CD4 + T-cells as a CTL Resistant Sanctuary for Intact HIV Proviruses

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Background: The distributions of defective and intact HIV proviruses differ between memory $CD4^+$ T-cells (Tm) and naïve $CD4^+$ T-cells (Tn). Tm generally contain substantially higher levels of total proviruses – the large majority of which are defective. In contrast, Tn contain less HIV DNA, but were recently reported to contain greater proportions of intact versus defective proviruses than Tm. Here we sought to validate these findings in an independent cohort, and to explore a potential role for differential intrinsic susceptibilities to CTL of Tn and Tm in giving rise to these skewed distributions.

Methods: Tn ($CD45RA^+$) and Tm ($CD45RA^-CCR7^+CD95^-$) were sorted from the PBMCs of ARV-suppressed donors by flow cytometry. Intact and defective proviruses were quantified by intact proviral DNA assay (IPDA). 'CTL-resistance assays': Ex vivo $CD4^+$ T-cells from HLA-$A02^+$ ARV-treated donors were subjected to strong CTL selection (independent of HIV expression), by pulsing with a CMV peptide and co-culturing with an HLA-A02-restricted CMV NV9-specific CTL clone. IPDAs were performed on survivors.

Results: In 9 ARV-treated donors, Tm and Tn contained medians of 1,282 and 131 copies of total HIV DNA/10^6 cells (ranges:Tm 95–3,222, Tn 6–488). However, in Tm a median of only 12% of these were intact, versus 40% in Tn (median:range, Tm-114: 0–712, Tn–54: 0–125 intact proviruses/10^6 cells, $p < 0.05$). In CTL resistance assays, Tn cells resisted killing relative to Tm, resulting in peptide-dose-dependent enrichments of Tn amongst survivors ($p = 0.01$, $n = 6$). In this initial cohort, the surviving cells (Tn-enriched) from CTL-resistance assays were confirmed by IPDA to have significantly higher intact:defective proviral ratios than $CD4^+$ T-cells that had not been selected by CTL (median 45% in survivors vs 13% in no-peptide control). In a second cohort, however, further dissection by enriching survivors into Tn and Tm populations (based on CD45RA) revealed heterogeneity. In some donors, intact proviruses were enriched within surviving Tn and Tm, while in others they were depleted.

Conclusions: By virtue of their intrinsic resistance to CTL, Tn may comprise a sanctuary for intact HIV proviruses. Within isolated Tn and Tm populations, however, whether cells harboring intact proviruses are disproportionately CTL resistant appears to vary by donor, requiring further investigation.

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PP 4.17 – 00198

Peripheral Blood Biomarkers of Occult Infection in ART-Suppressed, SIV-Infected Rhesus Macaques

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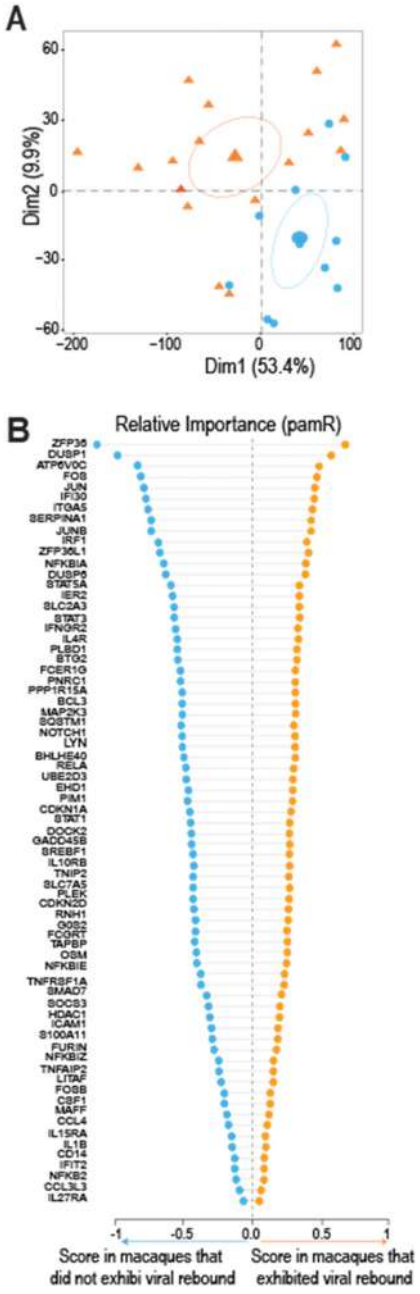
Background: A major barrier in the HIV-1 cure field is our incomplete knowledge of mechanisms regulating the establishment and maintenance of the viral reservoir.

Previous studies in SIV-infected rhesus macaques have suggested that the reservoir is seeded within three days before plasma viremia.

We initiated ART in rhesus macaques at 6 hours (Group 1), 1 day (Group 2), 2 days (Group 3), and 3 days (Group 4) after SIVmac251 infection.



All animals started ART before plasma viremia; thus, their infection status was unknown until ART was discontinued after six months of ART suppression.

Following ART discontinuation, we observed viral rebound in 0/5 animals in Group 1, 1/5 in Group 2, 3/5 in Group 3, and 5/5 in Group 4.

In this study, we used bulk transcriptomic sequencing and serum cytokines profiling combined with bioinformatic and machine learning analyses to identify potential peripheral blood biomarkers associated with occult infection and viral rebound following ART discontinuation.

Methods: We used bulk transcriptomic and plasma proteomics profiling to identify potential biomarkers of the rebound virus following treatment interruption.

We implemented a machine learning approach to identify and validate a list of biomarkers that predicted the rebound virus with an accuracy higher than 95%.

Results: We described a novel signature of occult SIV infection during ART suppression in macaques that initiated ART shortly after SIVmac251 challenge.

This signature involved immune activation and inflammatory pathways and accurately predicted viral rebound following ART discontinuation.

In macaques that exhibited viral rebound following ART discontinuation, we detected during ART suppression increased inflammatory pathways and T cell and monocyte activation signatures. Peripheral blood expression of proinflammatory cytokines (IL-8, IL-1RN, IL-1B, CCL4, and IL-15RA) were upregulated in macaques that exhibited viral rebound following ART discontinuation and correlated with the rebounding virus.

Conclusions: We showed that pro-inflammatory and immune activation signatures in peripheral blood and lymph nodes during ART suppression correlate with occult infection in SIV-infected macaques, as identified by viral rebound following ART discontinuation. If these biomarkers of the rebound-competent viral reservoir prove generalizable, they could be used in HIV-1 cure studies to identify promising interventions prior to analytic treatment interruption studies.

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PP 4.18 – 00213

Dynamics and antiviral role of TOX + TCF1 + CD39 + CD8 T cells in lymphoid tissue of SIV-infected rhesus macaques

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Background: CD8 T cell responses to HIV/SIV infection critically contribute to control of viremia, but lymph node (LN) CD8 T cells have been shown to be phenotypically and functionally distinct from and less cytolytic than those in blood.

Methods: To characterize the unique CD8 T cell populations in LN, we obtained LN from rhesus macaques at day 42 post-SIVmac239 infection and performed flow cytometry, scRNA-seq and imaging to characterize the dynamics of LN CD8 T-cells expressing Tox, TCF1 and CD39, three well-described markers

delineating exhausted, stem-like and terminally-differentiated populations.

Results: Tox is upregulated after SIV infection and primarily expressed in PD-1 + /TIGIT + cells. Notably, we observed high expression of Tox on a previously undescribed TCF1 + CD39 + population, distinct from TCF1 + CD39- stem-like and TCF1-CD39 + effector/exhausted CD8 T cells, that significantly expands after infection. These TCF1 + CD39 + cells expressed inhibitory receptors, intermediate levels of Ki-67, and are uniquely high in GzmK but low in GzmB, a profile consistent with a precursor effector cell. After SIV peptide stimulation, TCF1 + CD39 + cells degranulated at similar levels to TCF1-CD39 + cells but produce less IFNy. scRNA-seq of SIV-specific CD8 T cells revealed an intermediate profile of TCF1 + CD39 + cells between the stem-like TCF1 + CD39- cells and the differentiated TCF1-CD39 + cells. Importantly, a higher frequency of both Tox + and TCF1 + CD39 + CD8 T cells in LN at d42 p.i. was significantly associated with lower plasma viremia, lower levels of cell-associated SIV DNA [PM1] and better CD4 preservation. After 1 year of ART, the level of TCF1 + CD39 + LN CD8 T cells is associated with lower frequency of CD4 T cells harboring intact SIV-DNA. Investigations into potential mechanisms contributing to viral control revealed that TCF1 + CD39 + cells expressed elevated levels of CXCR5 compared to traditional effector cells, and expression of CXCR5 within TCF1 + CD39 + cells was associated with lower plasma viremia. Imaging analysis confirmed increased presence of TCF1 + Tox + CD8 T cells within B cell follicles compared to TCF1-Tox + and TCF1 + Tox- cells.

Conclusions: These data are consistent with a unique precursor effector CD8 T cell population that expands in LN after SIV infection, have a better ability to access the LN BCF, and is associated with increased viral control and reduced disease progression.

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PP 4.19 – 00021

Impact of SARS-COV-2-Mediated CD4 T Cell Activation HIV DNA Persistence In Vivo

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Background/Aims: Antigen-driven CD4 + T cell proliferation is a proposed mechanism of HIV-1 reservoir persistence. We previously reported that SARS-CoV-2 infection leads to increased detectable low-level HIV-1 plasma RNA blips months after COVID-19, but the impact of SARS-CoV-2-mediated T cell activation on expansion of HIV-1 reservoirs is not known. We sought to identify if SARS-CoV-2 infection leads to expansion of preferentially HIV-infected CD4 + T cells in people with HIV (PWH) on ART.

Methods: Five PWH with samples collected prior to and approximately two months after SARS-CoV-2 infection were identified. We performed a surface activation induced marker (AIM) assay using a CD4-optimized overlapping SARS-CoV-2 peptide pool to measure OX40/CD137 expression following peptide stimulation and sorted CD4 + T cells based on surface marker expression. ddPCR quantification of genomic HIV-1 DNA was performed on sorted subsets.

Results: We observed an increase in the frequency of SARS-CoV-2 AIM + non-naive CD4 + T cells following COVID-19 in samples from 4 of 5 participants (mean AIM + % 0.13 pre- vs 0.31 post). A large percentage of non-naive AIM + CD4 + T cells expressed PD1 compared with total non-naive cells before (76% vs 36%) and after (65% vs 19%) COVID-19; PD1 expression was lower following SARS-CoV-2 in both AIM + and AIM- CD4 + T cell subsets (though few were AIM + prior to COVID-19). HIV-1 DNA levels in non-naive AIM- CD4 + T cells prior to COVID-19 unexpectedly decreased following infection (mean 3,522 to 766 copies/10^6 cells). The numbers of AIM + cells obtained by cell sorting were overall low (3,863 mean) and only one participant had detectable DNA in post-COVID AIM + CD4 + T cells. However, a large majority of this participant's post-COVID AIM + cells harbored HIV-1 DNA (0.89 copies per cell) whereas HIV DNA in their AIM- cells decreased from 8,387 to not detected following SARS-CoV-2 infection.

Conclusion: COVID-19 in PWH led to a modest SARS-CoV-2-specific CD4 + cell response approximately two months following acute presentation. One participant may have preferentially expanded HIV-1-infected, SARS-CoV-2-specific CD4 + T cells following COVID-19 but future studies with more participants and numbers of cells will be needed to fully understand the impact of SARS-CoV-2 on clonal expansion and HIV persistence.

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PP 4.20 – 00025

A Metabolic Approach to Eradicate HIV brain viral reservoirs

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Background/Aims: Since the introduction of ART, HIV has become a chronic disease. The major obstacle to eradicating HIV infection is the presence of viral reservoirs (VRs). Upon peripherical infection, HIV integrates its viral DNA into the host chromosomes of microglia/macrophages and a small population of astrocytes. Few cells survive the acute infection to become long-lived VRs. However, the mechanisms of viral survival and latency are unknown. Here we show that the metabolism of the HIV latently infected cells is different from healthy cells and that mitochondria in HIV reservoirs are enlarged and excluded from autophagosomes, suggesting compromised fusion/fission and autophagy. Despite these alterations, no mitochondrial compromise was detected except for a large accumulation of alpha-ketoglutarate (αKG). Raise in the αKG levels correlated with viral silencing and latency. Here we identify the mechanisms of latency. Therefore, I hypothesize that "microglia and macrophages utilize the axis among mitochondria, autophagy, and nuclei to become latent.

Methods: Here, we characterized the metabolomics of four latently infected cell lines and primary cells. We treated the cells for 24 hours with TNF-α and α-KG and performed seahorse, western blot, immunofluorescence microscopy, PCR, and flow cytometry.

Results: α-KG is not only a TCA intermediary but also a transcription factor. α-KG alone can regulate HIV replication and silencing. We identified that primary cells with latent HIV integrated mostly use glutamate and glutamine as significant energy sources to produce ATP. This is significant because

glutamate is already dysregulated in the HIV CNS and the brain's more abundant neurotransmitter. Thus, viral reservoirs have an unlimited energy source to survive for an extended time.

Conclusion: First, we identified the metabolic pathways involved in the survival of VRs; second, we determined the role of mitochondria in the survival observed in VRs; and third, we demonstrated that VRs in vivo have metabolic alterations that could be targeted to eradicate them. In conclusion, we propose that combined metabolic changes could help kill VRs.

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PP 4.21 – 00050

Seeding of long-lived HIV cellular reservoirs through differentiation of infected CCR5 + CD4 + T cells into central memory cells

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Background/Aims: CCR5 is the main HIV coreceptor since CCR5 (R5)-tropic viruses are nearly always involved in the initial infection. Intriguingly, naïve cells do not express CCR5 and start to increase CCR5 expression as they proliferate upon stimulation. Once these cells are infected by HIV, some of these cells converted into long-lived memory cells which contribute to the HIV latent reservoir. Among these cells, central memory cells (TCMs) are essential to the stability of the reservoir because of

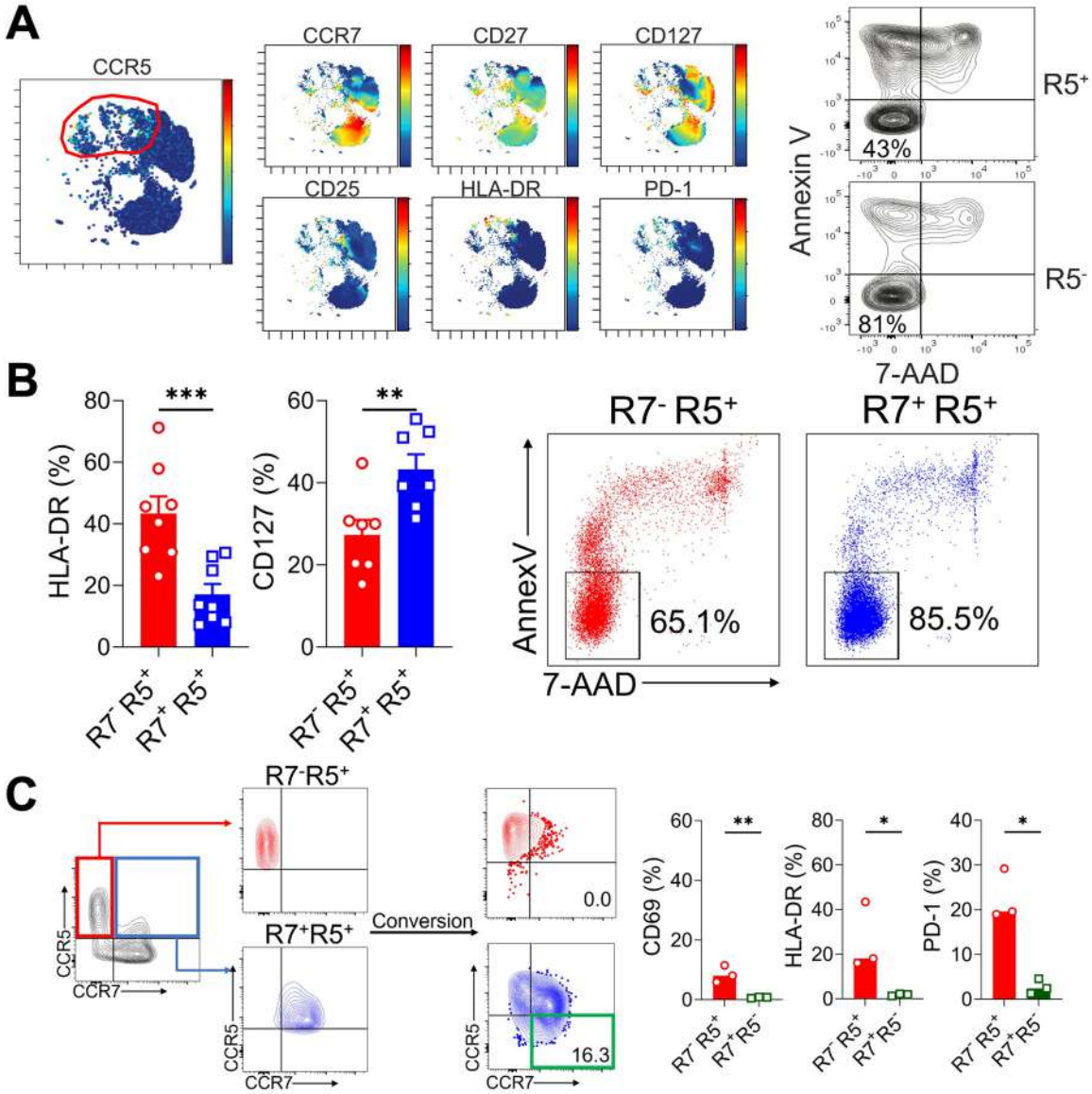

Figure 1. (A) CCR5⁺ cells displayed an effector cells phenotype and had shorter half-lives than CCR5⁻ cells. (B) CCR7⁺ CCR5⁺ cells had higher viability and more quiescence status than the CCR7⁻CCR5⁺ counterpart. (C) Some of infected CCR7⁺ CCR5⁺ cells lost CCR5 expression and became quiescent.

Figure 1 (abstract: PP 4.21–00050)

their self-renew capacity. In our study, we characterized how CCR5$^+$ CD4$^+$ T cells differentiate and convert into TCMs.

Methods: We used mass cytometry to characterize the activation and exhaustion status of CCR5$^+$ and CCR5$^-$ CD4$^+$ T cells. Their viability and proliferation capacity were determined to confirm the difference in phenotype between CCR5$^+$ and CCR5$^-$ cells in vitro. We found that CCR7$^+$ CCR5$^+$ effector cells had distinct phenotype from CCR7$^-$ CCR5$^+$ cells after stimulation. Next, we compared these two groups of cells in viability, susceptibility to CCR5-tropic HIV, and the conversion into quiescence status in vitro and vivo.

Results: We showed that CCR5$^+$ cells displayed an effector cells phenotype with much higher levels of expression of CD25, HLA-DR, PD-1, and CTLA-4 compared to CCR5$^-$ cells, and had lower proliferation capacity and shorter half-lives than CCR5$^-$ cells. However, among CCR5$^+$ cells, there was a rapid emergence of a small subset of CCR7$^+$ CCR5$^+$ cells with comparable susceptibility to HIV. After HIV infection, some of the CCR7$^+$ CCR5$^+$ cells lost CCR5 expression. This group of cells had higher viability and more quiescence status than the CCR7$^-$ CCR5$^+$ counterpart, and phenotypically resembled central memory reservoirs (Figure 1).

Conclusion: Although most CCR5$^+$ CD4$^+$ T cells are short-lived effector cells, a small fraction of CCR5$^+$ cells can turn on CCR7 expression and convert into quiescent memory-like cells. Our study models how HIV-infected cells become long-lived memory cells, which helps to better understand the formation of the stable latent HIV reservoirs.

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PP 4.22 – 00087

Reversal of exhaustion of HIV-1-specific CTLs by CRISPR-mediated disruption of PD-1 gene

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Background/Aims: CTLs (cytotoxic T lymphocytes) mediate strong suppression of HIV infection. Unfortunately, chronic HIV-1 infection drives functional exhaustion of viral antigen-specific CTLs with elevated and sustained expression of the immune checkpoint receptors such as PD-1. This functional impairment, including lack of antigen-driven proliferation and poor cytolytic capacity (Fig. 1A), cannot be fully restored by ART (antiretroviral therapy). CRISPR-Cas9 technology allows specific and efficient editing of human genes and is widely used to knockout endogenous TCR gene expression in CAR-T cells for cancer immunotherapy. Here we developed and validated a CRISPR-Cas9-based strategy to knockout PD-1 gene expression in CTLs.

Methods: The human PDCD1 (PD-1) gene coding sequence was screened using the CRISPR design tool (CRISPOR) for the presence of gRNAs binding sites for AAV-compatible SaCas9 (NNGRRT PAM sequence). A set of five gRNAs targeting exons 1–3 of the PDCD1 gene having the highest ON-target efficiency and the lowest OFF-target scores were selected. Next, candidate gRNAs were tested by electroporating ribonucleoprotein complexes composed of recombinant SaCas9 and synthetic gRNAs into the Jurkat T-lymphoid cell line. The most efficient gRNA combination was validated in experiments using primary CD8+ T cells from three healthy donors and people living with HIV (PLWH). CRISPR-mediated excision of the PDCD1 gene was evaluated by PCR genotyping and Sanger sequencing (Fig. 1B,C).

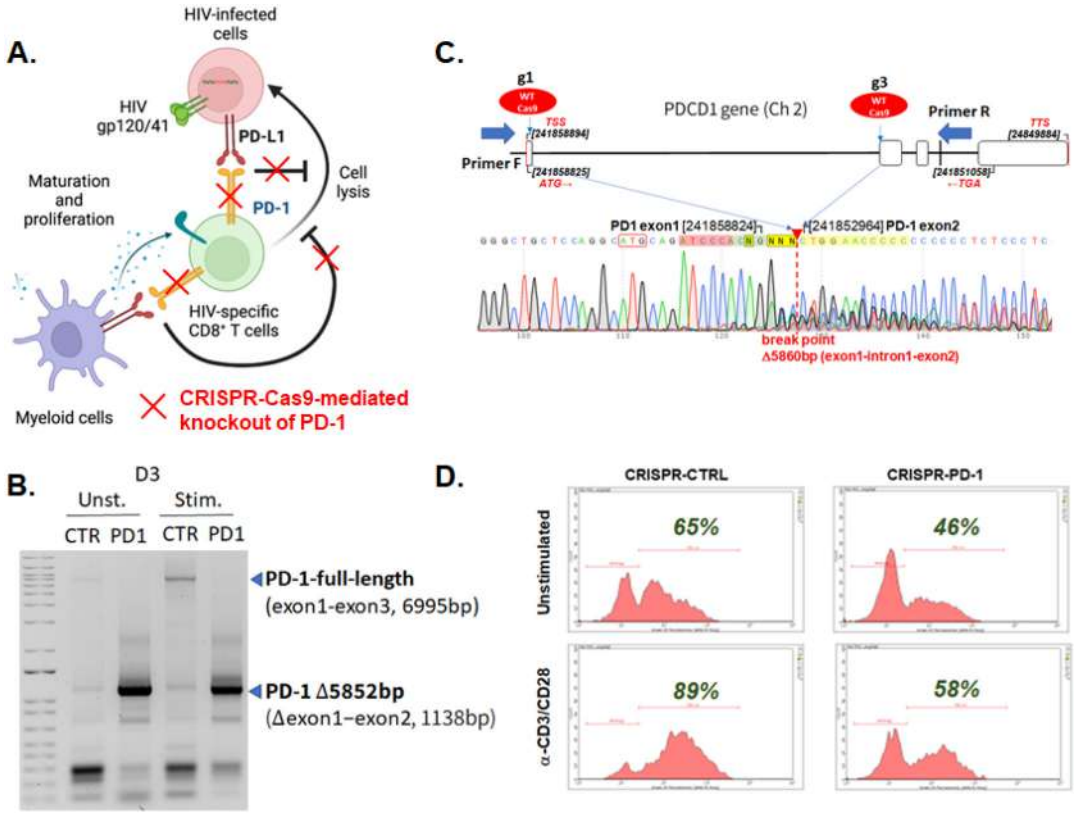

Figure 1 (abstract: PP 4.22–00087) CRISPR Mediated Knockout of PD-1 in CTLs

Cell surface expression of PD-1 was checked by immunolabeling/flow cytometry (Fig. 1D). Finally, the immunoproliferation of control and CRISPR-PD-1 treated CD8+ T cells was examined upon co-culture with isogenic monocyte-derived macrophages (MDMs) in the presence of an HIV-1 gag-peptide pool.

Results: A pair of SaCas9-specific gRNAs targeting exon1 and exon3 of the human PDCD1 gene allowing robust and repeatable knockout of PD-1 expression in T-lymphoid cell line and primary CD8+ T lymphocytes, was identified. In addition, AAV-delivery "all in" vector carrying SaCas9, and two PD-1 specific gRNAs was constructed and tested.

Conclusion: The CRISPR-SaCas9 platform can be used for specific and efficient knockout of PD-1 expression in CTLs. Furthermore, generated AAV-CRISPR-PD-1 vector enables future in vivo studies.

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PP 4.23 – 00148

Inhibition of the GSK3 pathway enhances CD8+ T cell stemness and functional capacities without promoting non-cytolytic suppression of HIV transcription

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Background/Aims: HIV persists indefinitely in infected individuals despite fully suppressive antiretroviral therapy (ART) due to a reservoir of latently infected cells. Previously we have demonstrated that, in addition to antigen-specific responses, CD8+ T cells can suppress HIV replication through a non-cytolytic mechanism resulting in inhibition of virus transcription. Recent studies have shown that targeting the Wnt signaling pathway in CD8+ T cells through GSK3 inhibition promoted stemness and functionality associated with natural control of HIV infection. Here we determined the impact of CD8+ T cell reprogramming on the non-cytolytic suppression of HIV expression in CD4+ T cells.

Methods: CD8+ T cells from HIV-naïve donors were treated for 16 hours with 3uM GSK-3 inhibitor (BIO), vehicle control or medium. After reprogramming, CD8+ T cells were TCR activated for 48 hours, and then co-cultured for 3 days in the presence of ART with autologous memory CD4+ T cells in vitro infected with HIV. CD8+ T cell suppression activity was quantified as HIV-gag expression by flow cytometry and integrated HIV DNA frequency by qPCR. The phenotypic profiles of CD8+ and CD4+ T cells were characterized by flow cytometry.

Results: Treatment of CD8+ T cells with BIO enhanced the expression of TCF-1 and promoted the enrichment of the central memory and stem cell memory phenotype. When co-cultured with HIV-infected CD4+ T cells, reprogrammed CD8+ T cells exhibited lower levels of the exhaustion markers PD-1, TIGIT, and TIM3, and higher levels of the degranulation marker CD107a. As we have shown previously, HIV expression in memory CD4+ T cells was reduced when co-cultured with CD8+ T cells as compared to CD4+ T cell monocultures, without a reduction of the frequency of HIV-infected cells; interestingly, metabolic reprogramming through GSK3 inhibition did not enhance CD8-mediated suppression activity. However, when co-cultured with reprogrammed CD8+ T cells, HIV-infected-CD4+ T cells showed a trend toward higher levels of activation and higher expression of BCL-6, T-bet, and BLIMP-1.

Conclusion: Our results show that inhibition of GSK3 pathway could be a useful tool to enhance stemness and functional properties of CD8s, boosting their antiviral potential, without enhancing the non-cytolytic suppression of HIV expression.

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PP 4.24 – 00176

Impact of cannabis use on immune cell populations and the viral reservoir in HIV-infected people on suppressive antiretroviral therapy

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Background: HIV infection remains incurable due to the persistence of a viral reservoir during antiretroviral therapy. Cannabis (CB) use is prevalent amongst people with HIV (PWH), but the impact of CBs on the latent HIV reservoir has not been investigated. We hypothesized that CB exposure during HIV infection alters the size and phenotype of the latent HIV reservoir through the activation of CB-dependent signaling in CD4 T cells.

Methods: Peripheral CD4 and CD8 T cells from a cohort of CB using PWH and a matched cohort of non-users were analyzed by flow cytometry for expression of maturation, activation, and functional markers and with an intact proviral DNA assay.

Results: CB use was associated with significantly increased abundance of naïve T cells, reduced levels of effector cells, and with reduced expression of activation markers within both CD4 T cells and CD8 T cells. Furthermore, CB users exhibited significantly reduced levels of exhausted and senescent T cells compared to non-using controls. HIV-specific T cell responses were unaffected by CB use. A trend towards a lower frequency (~2 fold) of intact HIV proviruses was observed in CB users, although this difference was not statistically significant across the whole population ($P = 0.2$). However, a significant impact of CB use on intact reservoir size was observed in participants with a high frequency of CD16 or NKG2A expression in NK cells.

Conclusion: Overall, this analysis indicates that CB use reduces activation, exhaustion and senescence in the T cells of PWH and may have an effect on reservoir size. Further studies will be needed to clarify the impact of CB use on the HIV reservoir.

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PP 4.25 – 00196

The Role of Epigenetics in Mediating Neuronal Circuitry and Maladaptive Neuronal Changes in HIV and Opioid Drug Addiction

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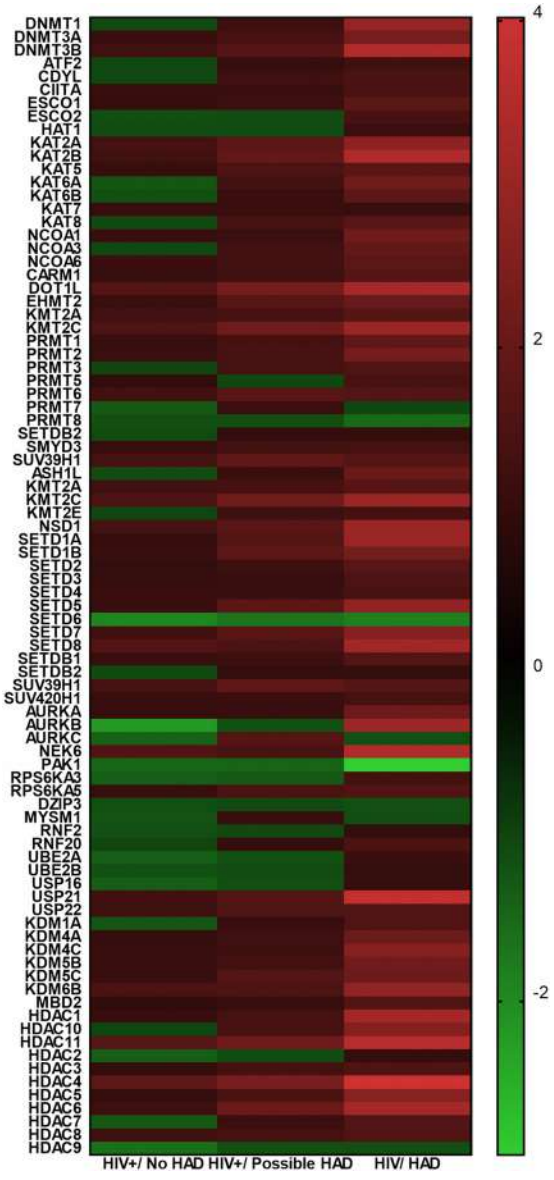
Background/Aims: Long-term and repeated exposure to illicit drugs induces alterations to areas in the brain involved with reward processing and motivation which leaves individuals susceptible to engage in pathological drug-seeking and drug-taking that can persist for a lifetime. To add insult to injury, there is a high prevalence of substance abuse among HIV-infected individuals. Substance abuse affects the delivery and outcomes of HIV medical management. Recent investigations have started to reveal potential genes that contribute to the risk for addiction, and it has been established that epigenetic mechanisms facilitate some of the lasting effects of drugs of abuse on the brain in animal models of addiction. My lab previously found that co-exposure of the opiate, morphine, with combined antiretroviral drugs (cART) negated the inhibitory effects of cART, leading to increased viral load and increased secretion of inflammatory molecules. Genetic analysis revealed a significant increase in the expression of histone-modifying enzymes contributing to the neuropathology associated with HIV expression of histone-modifying enzymes and G-protein coupled receptors in HIV-infected brain cells. The aims of this study are as follows: (1) investigating changes in expression levels of chromatin modifying enzymes potentially associated with HIV-pathology and drug seeking behavior using post-mortem brain tissue samples of HIV-infected patients with a history of opioid/polysubstance abuse, and/or neurological impairment and (2) investigating and comparing potential changes in expression levels of chromatin modifying enzymes potentially associated with HIV-pathology and drug seeking behavior using PBMC samples of HIV-infected patients with a history of opioid/polysubstance abuse, and/or neurological impairment.

Methods: Qiagen's RT2 profiler PCR array for human epigenetic chromatin enzymes, a highly sensitive and reliable method for gene expression analysis, was used.

Results: There was an increase in fold change of chromatin modification enzyme expression relative to the control group as neurological impairment worsened in postmotem brains. The greatest increases are seen in DNA/Histone Demethylases, Histone Deacetylases/Acetyltransferases, and DNA/Histone Methyltransferases.



Conclusion: Since a general picture of genetic and epigenetic mechanisms involved in HIV pathology and drug addiction is beginning to emerge, the insight gained from these studies will be key to identifying novel targets in HIV-1-opioid crosstalk for improved diagnosis and treatment.

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PP 4.26 – 00201

Study of reservoir size and telomere length in children with perinatal HIV-1 infection who achieved late viral suppression

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Background/Aims: Children with HIV-1 who start highly active antiretroviral therapy (HAART) early, have a better prognosis for immune recovery and smaller reservoir size. However, long-term infection and HAART can lead to the development of

comorbidities associated with premature immunosenescence. There are few data on the inter-relationship between the size of the viral reservoir and immunosenescence.

Our objective was analyze the association between HIV-1 reservoir size and absolute telomere length (aTL) in children who acquired the infection perinatally, achieved sustained viral suppression (SVS) (<400 copies of HIV-RNA/ml) at advanced ages (≥6 years) and maintained it for at least 3 years.

Methods: This is a cross-sectional analytical study; the patients were born in the period 2000–2007 in Argentina and were under follow-up. From total mononuclear cells (PBMC) aTL and reservoir size –total HIV-DNA- were measured by quantitative real-time PCR (qPCR) with SYBR Green I and semi-nested qPCR with Taqman probes, respectively. *Poisson* linear regression was used. We also studied the association with % $TCD4^+$, % $TCD8^+$, age of initiation and time on HAART and SVS. Reservoir, aLT, time on HAART and SVS were evaluated as continuous variables. Values were expressed as medians and interquartile ranges.

Results: Fourteen patients with a median age of 15 (14–17) years, 12 (8–14) years on HAART, and 6 (4–7) years on SVS were evaluated. The aTL median was 162 (150–180) Kb/genome and the reservoir was 4,05 Log_{10} copies HIV-1/10^6 PBMC (3,9–4,1). The aTL was associated positively with time on HAART (R^2 = 0,40 p < 0,01), and the reservoir was associated negatively with time on SVS (R^2 = 0,39 p < 0,01). Both models improved with the inclusion of the % of $TCD8^+$, the R^2 was 0,61 (p < 0,01) for the reservoir, and the aTL, adjusted for age, the R^2 was 0,60 (p < 0,01). No association was found between aTL and reservoir size.

Conclusion: We did not find an association between the size of the reservoir and aTL, however, our results suggest that a long time on HAART has a beneficial effect on immune recovery, represented by a longer aTL. This study highlights the need not only to achieve early initiation of HAART but also to achieve SVS.

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PP 4.27 – 00202

Single-cell RNA sequencing reveals CBD genetic signature in monocyte gene expression

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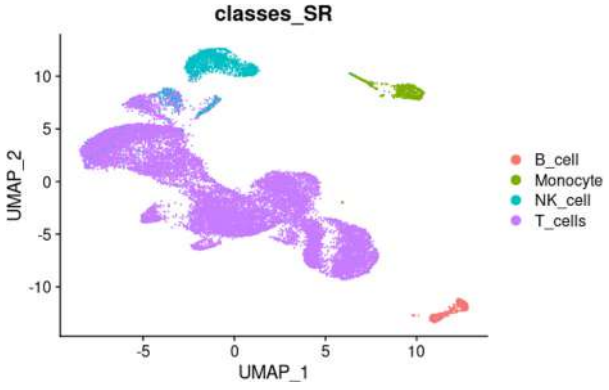
Background/Aims: HIV-related comorbidities appear to be strongly related to chronic inflammation, a condition characterizing PLWH. Prior work showed that cannabidiol (CBD) treatment during human monocyte differentiation reduces both macrophage susceptibility to HIV infection, and systemic. CBD also has anti-inflammatory properties, however, its cellular mechanisms used to alter inflammation are poorly understood.

Our objective is to detect gene expression alterations in peripheral blood mononuclear cells (PBMCs) from PLWH blood samples after 1-to-2 months of CBD exposure.

Methods: We collected blood samples from two PLWH volunteers at baseline, after 1 month, and 2 months of CBD administration. Enrolled subjects were negative to drug tests, did not take anti-inflammatory medications, and were instructed to take sublingual CBD twice per day, 12 hours apart (morning and evening, 62.5 mg/day). Samples were collected at UF Clinical Research Center. PBMCs were extracted from fresh samples, cryopreserved, and processed (library preparation and sequencing via NovaSeq 6000) according to the recommended 10X PBMC protocol. Downstream analysis was conducted with Surat and SingleR. Differential gene expression was measured through a negative binomial model, with thresholds of 1 on log2FC, and 0.05 on p-values.

Results: After quality filtering, we obtained ~20,000 cells: B cells (n = 432), monocytes (579), NK cells (1,915), and T cells (17,538); together expressing a total of ~38,000 distinct genes. In monocytes, we found CBD significantly alters the expression of TEX14 (log2FC 1.3, p-val 6.4e-10), WDR74 (log2FC 1.19, p-val 2.3e-17),SLC2A3, (log2FC 1.2, p-val 4.3e-05), ID2 (log2FC 1.08, p-val 6.8e-10) and JUN (log2FC 1.1, p-val 9.1e-10); in B cells, IGHG (log2FC 2.2, p-val,0.037). No significant gene expression pattern variation was detected in NK and T cells. Both WDR74, JUN, and SLC2A3 are related to the alternative (i.e. anti-inflammatory) macrophage phenotype; and ID2 is a tissue-specific environmental driver macrophage and monocyte differentiation.



Conclusion: Our study shows how CBD induces cell-specific alterations of gene expression in monocytes and B cells after an exposure of 1-to-2 months. Detecting these changes is the first step towards the understanding of CBD molecular mechanics and its interaction with PBMCs.

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Session 5: Drug Discovery Development & Pharmacology

OP 5.2 – 00070

Characterization of a dual PTPN1/PTPN2 inhibitor to target latent HIV reservoirs

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Background: 3-Hydroxy-1,2,3-Benzotriazin-4(3H)-one (HODHBt) is a latency-reversal agent (LRA) that enhances γc-cytokine signaling by increasing phosphorylation and transcriptional activity of STAT5. We have shown that HODHBt increases IL-2 and IL-15 activation of STAT5, promoting reactivation from latency in a primary cell model and cells isolated from people living with HIV. Furthermore, we have recently shown that HODHBt enhances IL-15 mediated NK effector function. In here, we aimed to characterize HODHBt cellular target to further develop small molecules that can be used to reduce latent reservoirs.

Methods: We first used cellular thermal shift assay followed by mass-spectrometry (CETSA-MS) to identify binding proteins of HODHBt in peripheral blood mononuclear and K562 cells. Second, we used STRING pathway analysis to identify known interactions of the identified proteins with STAT5. We then used CRISPR/Cas9 to confirm the role of these proteins in STAT5 phosphorylation and transcriptional activity. Next, we used an *in vitro* assay to demonstrate the role of HODHBt inhibiting the enzymatic activity of the targets. Finally, we evaluated the effects of HODHBt in CD8 T cells and γδ-T cells phenotype and function.

Results: CETSA-MS analysis identified 11 HODHBt-interacting proteins including the non-receptor phosphatases PTPN1 and PTPN2 in both cell types (Adj. $p < 0.01$). CETSA followed by western blot confirmed changes in thermal stability of PTPN1 and PTPN2 in the presence of HODHBt. CRISPR/Cas9 confirmed that knock-out of both PTPN1 and PTPN2 increased STAT5 phosphorylation and transcriptional activity in a synergistic

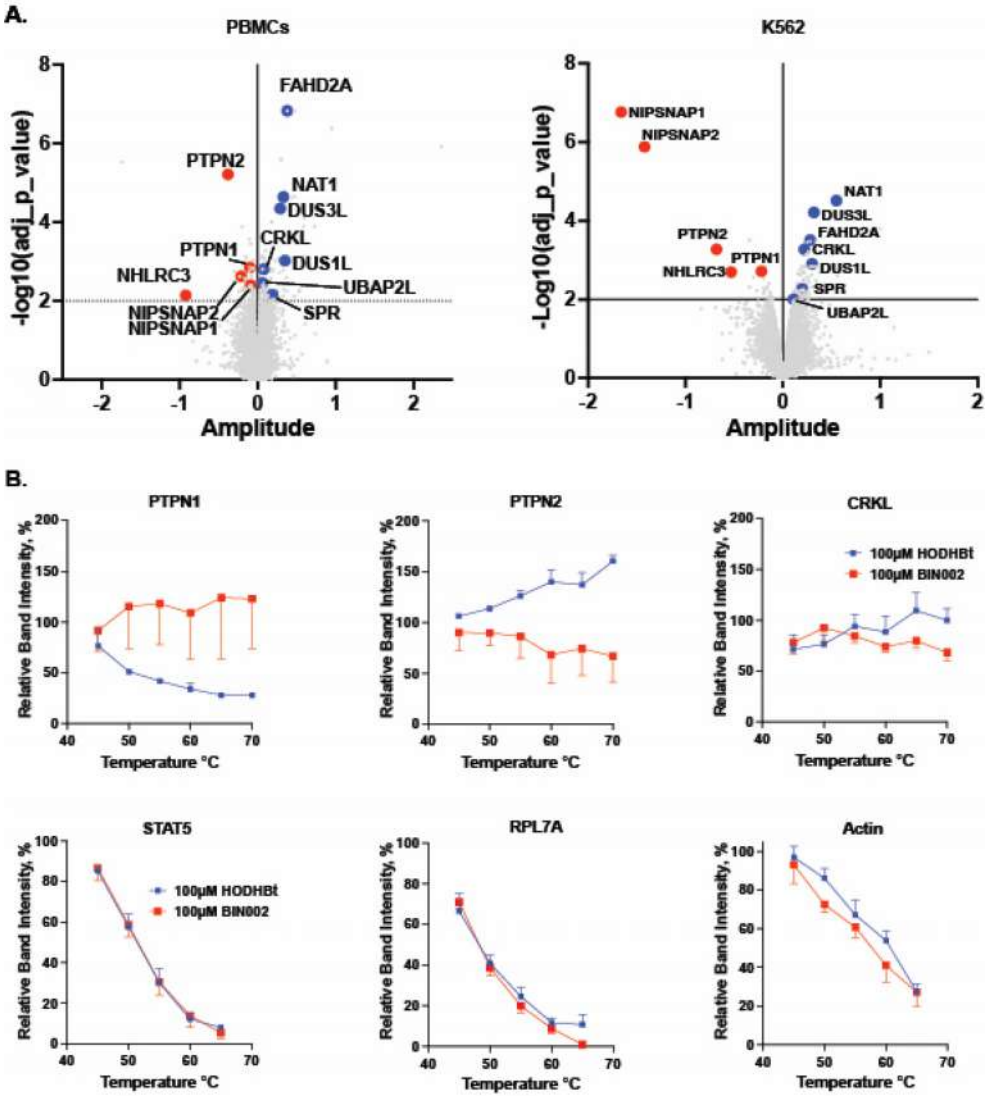

Figure 1 (abstract: OP 5.2–00070) Identification of PTPN1/PTPN2 as HODHBt targets.

manner. Mechanistically, HODHBt inhibited the catalytic domain of both PTPN1 and PTPN2 and promoted the degradation of PTPN2. Finally, we demonstrated that HODHBt enhanced cytokine mediated expansion of CD8 T cells by 2-fold over a 21-day culture period (n = 6, Wilcoxon p = 0.031), and increased the expression of Granzyme B in CD8 T cells (n = 9, Wilcoxon p = 0.011) and γδ-T cells (n = 1).

Conclusions: We have identified PTPN1 and PTPN2 as the targets of HODHBt. PTPN1 and PTPN2 are intracellular checkpoints that limits cytokine-mediated T cell responses. Our results demonstrate that inhibiting PTPN1/PTPN2 enhanced latency reversal and immune effector functions. The development of PTPN1/PTPN2 dual inhibitors could be an attractive approach towards HIV eradication either alone or in combination with other immune-based strategies.

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OP 5.3 – 00082

1-year treatment with ponatinib provides protection of CD4 + T cells against HIV that is maintained at least 1 year more after treatment interruption

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Background: PLWH treated with ART and dasatinib show reduced reservoir size that is resistant to reactivation. Now we determined if treatment with ponatinib may also protect CD4 from HIV infection during treatment and if this protection was maintained after treatment interruption.

Methods: 9 participants of multicenter, open-label, single-arm, Phase II exploratory trial NCT04043676 were recruited. They achieved deep molecular response against chronic myeloid leukemia (CML) after 14 (IQR 5.5–15.5) years of treatment with imatinib before interruption and then received 1 year-consolidation treatment with ponatinib 15 mg/day. Blood samples were taken before starting ponatinib, after 1 year-treatment, and 3, 6 and 12 months after interruption to monitor CML relapse. Activated PBMCs were infected with NL4-3_wt 72 h. HIV-p24 core antigen, SAMHD1 phosphorylation at T592 (pSAMHD1), CD4 memory and cytotoxic cell populations were analyzed by flow cytometry. PBMCs antiviral activity was evaluated by measuring caspase-3 activity in NL4.3_wt-infected TZM-bl cells.

Results: (1) 5 participants (55.5%) did not relapse from CML 12 months after ponatinib interruption (Non-relapsed); 4 participants (44.4%) relapsed after 5.5 months (IQR 4.25–6.75) of ponatinib interruption (Relapsed). (2) CD4 were susceptible to HIV infection in all participants while treated with imatinib; 1 year-treatment with ponatinib reduced 8.8-fold HIV infection (Figure 1a). This protection was maintained at least for 12 months of treatment-free remission (TFR) in Non-relapsed (p = 0.0039), which correlated with pSAMHD1 interference. (3) After CML relapse and imatinib reintroduction, all CD4 memory subpopulations regained susceptibility to HIV infection. (4) Antiviral cytotoxicity increased 2-fold (p = 0.0317) in PBMCs from Non-relapsed after 1-year of ponatinib (Figure 1b). Cytotoxic activity remained increased in Non-relapsed for 11 months of TFR. 5) CD8 degranulation activity (CD107a+) increased 4.08-fold (p = 0.0317) 3-months after interruption in Non-relapsed. Levels of TCRgd + cells increased in both groups (p = 0.0330) during TFR; CD107a expression increased 3.3-fold (p = 0.0078) after ponatinib withdrawal in Non-relapsed.

Conclusions: One-year treatment with ponatinib preserved SAMHD1 in CD4 and induced a potent sustained cytotoxic effect, impeding HIV infection and the formation of the viral reservoir. The antiviral protection was maintained at least 12 months during TFR in correlation with sustained antileukemic response. Short-term intensification treatment with TKIs could be useful for HIV cure strategies.

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OP 5.4 – 00127

Identification and characterization of novel inhibitors of HIV Tat protein

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Background: The HIV Tat protein is essential for the amplification of viral genome transcription and represents a potential antiviral target. Tat binds HIV mRNA's stem-bulge loop structure, the transactivation-responsive (TAR) element, to activate transcription. Block-and-lock approaches to cure HIV take advantage of HIV transcriptional inhibitors to limit viral rebound upon treatment interruption by promoting long-lasting epigenetic silencing. The Tat inhibitor, didehydro-cortistatin A (dCA), was shown to promote long-lasting epigenetic suppression of the HIV promoter (LTR). The cost and difficulty of dCA synthesis has prompted the search for less complex inhibitors, with exceptional drug-like properties, yet easier and cheaper to produce.

Methods: The HeLa-CD4 cell-based Tat-transactivation assay of the HIV LTR driving luciferase was used for high throughput screening (HTS) of 579 443 small molecules with dCA as a control. We followed hits with appropriate counter-screens and validation in physiologically relevant cells. Hit specificity was confirmed in multiple ways, including with Tat-TAR defective viruses. Molecular docking studies and Tat point mutations defined their binding site. The 26S proteasomal pathway engagement was identified using competitive inhibitors and shRNA knockdown of the E3 ubiquitin ligase Cereblon.

Results: We identified *via* HTS three compounds with a therapeutic index (TI) varying from 5 to 181 with good chemical properties. These leads inhibited acute HIV replication in multiple cell models and in human PBMCs, without affecting

cell viability. Moreover, these blocked viral reactivation in cell line models of HIV latency demonstrating their potential as block-and-lock agents. We demonstrated these compounds specifically promote degradation of Tat in multiple systems. The compounds were shown to act as molecular glues promoting Tat ubiquitination by the E3 ubiquitin ligase Cereblon that preceded degradation by the 26*S* proteasomal pathway. Computational molecular dynamics of their interaction with Tat as well as Tat mutant analysis revealed preferential binding to the conserved Tat residue Y26. Synthesis of novel analogs, structure-activity relationship studies, and pharmacokinetic studies are ongoing.

Conclusions: We identified three novel small molecules that define a unique class of anti-HIV drugs that inhibit Tat *via* engagement of proteasomal degradation. Protein degradation may prolong efficacy, reduce viral resistance, and block a broader range of Tat's pleiotropic activities.

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OP 5.5 – 00083

Impairment of HIV proviral reactivation by interfering with essential metabolic pathways in effector memory CD4 + T cells

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Background: HIV selectively infects highly metabolic CD4 + T cells. CD4 TEM and TEMRA subpopulations, which are essential for the reservoir replenishment, show the highest metabolic activity in comparison with naïve (TN) and TCM. Dasatinib is a potent cytostatic drug that interferes with HIV infection in CD4 and macrophages. We evaluated if dasatinib may be used as a latency promoting agent (LPA) by reducing the metabolic activity in viable CD4 cells to interfere with the replenishment of HIV reservoir by TEM and TEMRA reactivation without affecting other essential cells such as CD8 and NK cells.

Methods: CD4 + T cells from healthy donors were activated with PHA/IL-2 ± dasatinib 75 nM 72 h. PLWH on ART and dasatinib were also recruited. Phosphoproteome was analyzed by LC-MS/MS. Synthesis of mitochondrial ATP and changes in culture medium pH were analyzed every 24 h. Uptake of fluorescent glucose analog 2-NBDG, GLUT-1expression, distribution of T-cell memory subpopulations, and T-cell viability were determined by flow cytometry.

Results: (1) Dasatinib modified phosphorylation of >130 proteins involved in metabolic pathways such as glycolysis/glyconeogenesis, pyruvate metabolism, pentose phosphate pathway, inositol phosphate metabolism, phosphatidylinositol signaling system, purine metabolism, and biosynthesis of amino acids. (2) Dasatinib reduced 2.1-fold ($p = 0.0112$) the synthesis of mitochondrial ATP in viable CD4 cells in response to PHA/IL-2, which correlated with reduced susceptibility to HIV infection. (3) Culture medium pH remained stable at 7.6 in the presence of dasatinib after 72 h of activation but diminished to 7.2 without dasatinib. (4) Dasatinib interfered with GLUT-1 expression and glucose uptake induced by PHA/IL2 in all CD4 memory subpopulations, including TEM, and TEMRA (Figure 1); NK and CD8 cells remained metabolically active. (5) PLWH on ART and dasatinib showed reduced CD4 TEM and TEMRA subpopulations, in accordance with decreased metabolic activity in these cells.

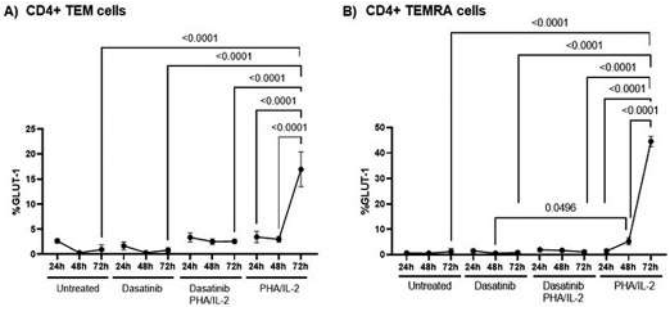

Figure 1

Conclusions: Dasatinib may act as LPA by selectively relegating viable CD4 cells to a resting state in which glycolysis and mitochondrial ATP synthesis were stalled, impeding both HIV infection and reservoir reactivation and replenishment, whereas CD8 and NK cells were not affected. Treatment with dasatinib along with ART may contribute to silence the viral reservoir as part of block&lock strategy.

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PP 5.1 – 00045

Impact of Intrinsic and Extrinsic Factors on the Pharmacokinetics of Long-Acting Lenacapavir for Treatment of HIV

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Background: Lenacapavir (LEN) is a novel, first-in-class selective inhibitor of HIV-1 capsid protein, currently being investigated in heavily treatment experienced (HTE) participants with HIV-1 (PWH). The ongoing Phase 2/3 studies in PWH uses every 6 months subcutaneous (SC) dosing with oral loading/lead-in (oral LEN 600 mg on Days 1 and 2, and oral LEN 300 mg on Day 8 followed by SC LEN 927 mg on Day 15 and every 6 months thereafter). The objective was to characterize the population pharmacokinetics (PopPK) of LEN and evaluate the effect of intrinsic/extrinsic factors that may affect LEN exposures.

Methods: Pharmacokinetic (PK) data were pooled from 7 studies in participants with and without HIV who received intravenous/oral/SC LEN. A total of 6855 LEN concentrations from 384 participants were analyzed in the PopPK analysis using nonlinear mixed effects modeling. Several intrinsic and extrinsic

factors/covariates including pharmacoenhancers (cobicistat or ritonavir), body weight (BW), age, sex, race, ethnicity, dose, disease status, food, formulation and estimated glomerular filtration rate were evaluated. LEN exposures were simulated using the bayesian posthoc PK parameters and presented across applicable covariates.

Results: A 2-compartment model with 1st order process for oral absorption, with a parallel 1st and transit compartment absorption for SC and linear elimination, adequately described LEN concentration data. The typical total clearance (CL), intercompartmental CL, central volume, and peripheral volume values were 4.05 L/h, 41.2 L/h, 68 L and 908 L, respectively. Dose was found to affect oral bioavailability and CL of LEN. HTE participants had lower CL (23.3% decrease) compared to participants without HIV. Pharmacoenhancers were found to affect oral LEN bioavailability (58.7% increase). The change in LEN exposures with BW ranged from approximately −32.3% to +23.5% (relative to the median exposures) for participants with 5th to 95th BW percentiles, respectively. No additional covariates were found to significantly affect LEN exposure.

Conclusions: Higher LEN exposures were observed with lower body weights and for participants on pharmacoenhancers. In addition, higher exposures were observed in HTE participants compared to participants without HIV, potentially due to unaccounted and complex disease- related cofounders. These changes in LEN exposures were not considered clinically meaningful.

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PP 5.2 – 00072

Simulations for Once Weekly Dosing of Oral Lenacapavir

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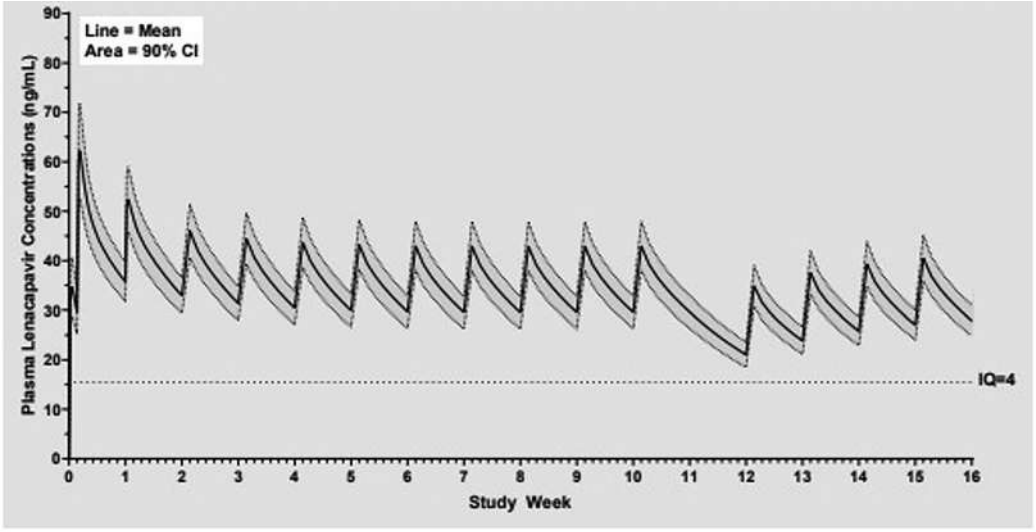
Background: Lenacapavir (LEN), a potent first-in-class inhibitor of HIV-1 capsid function, is in development for the treatment and prevention of HIV-1 infection. Current data indicates that LEN exhibits near maximal antiviral activity when the lower bound of the 90% confidence interval (CI) of mean Ctrough are maintained above inhibitory quotient 4 (IQ4) (at least 4-fold greater than the in vitro protein adjusted 95% effective concentration determined by MT-4 cells). LEN has potential to be used in combination with other antiretroviral agents for treatment and prevention of HIV-1 infection. The objective of this analysis was to utilize a previously developed population pharmacokinetic (PopPK) model of LEN to simulate various oral weekly dosing regimens that would rapidly achieve and maintain LEN concentrations above IQ4.

Methods: A 2-compartment PopPK model with first order absorption and linear elimination was previously developed to describe LEN plasma concentration data from multiple Phase 1, 2 and 2/3 studies (total 384 participants). This model was utilized to simulate various dosing regimens (loading doses + maintenance doses) that can achieve efficacious LEN concentrations rapidly and maintain it throughout the dosing interval. Additionally, various scenarios of missed oral doses were simulated to evaluate the forgiveness window. These simulations were performed with PopPK model incorporating the variability and covariate effects.

Results: Simulations showed that an oral loading dose of 600 mg on day 1 and day 2 followed by 300 mg oral once weekly doses maintained the lower bound of the 90% CI of mean Ctrough above IQ4 (15.5 ng/mL) throughout the dosing interval (Figure 1). This dosing regimen reached IQ4 rapidly within 4 hours of dosing initiation. In addition, simulations also showed that oral LEN administered once weekly is expected to maintain



*Profile shows 600 mg oral LEN dose on day 1 and day 2 followed by oral LEN 300 mg on day 8 and once weekly thereafter with a missed 300 mg oral dose at week 11 and continuation of 300 mg once weekly from week 12 onwards

Figure 1 (abstract: PP 5.2–00072) Oral Lenacapavir.

concentrations above IQ4 with a forgiveness window of up to 7 days after the last missed oral dose.

Conclusions: Once weekly oral LEN 300 mg is expected to maintain concentrations above IQ4 throughout the dosing interval while allowing for a 7-day forgiveness window after the last missed oral dose. Oral LEN can be developed as part of a complete oral weekly regimen for the treatment of HIV-1 infection.

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PP 5.3 – 00115

Chemical inhibition of DPP9 sensitizes CARD8 inflammasomes in HIV-1-infected cells

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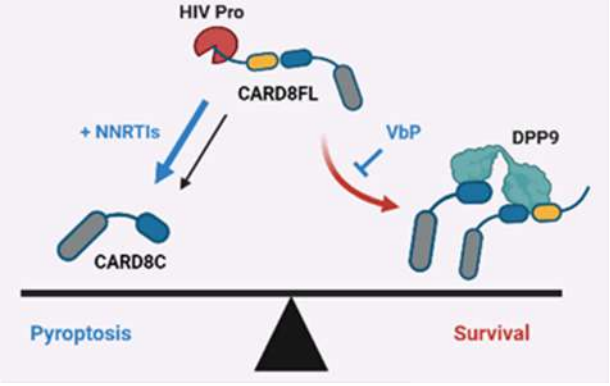
Background: The inflammasome sensor CARD8 can sense intracellular HIV-1 protease activity which leads to targeted cell death of HIV-1 infected cells. This premature intracellular activation can be achieved using NNRTIs. However, high concentrations of NNRTIs are required for sufficient CARD8 sensing. This calls for the elucidation of ways to sensitize the CARD8 inflammasome. This can be achieved through inhibition of the CARD8 negative regulator DPP9. The DPP9 chemical inhibitor Val-boroPro (VbP) can activate the CARD8 inflammasome in CD4+ T cells and was tested for its ability to enhance CARD8 inflammasome sensing of HIV.

Methods: CD4+ T cells were isolated from PBMC of healthy donors, infected with a pNL4-3-GFP reporter virus, and treated with NNRTIs with or without VbP. Site-directed mutagenesis via PCR was used to introduce NNRTI resistance associated mutations. To test the combination strategy in vivo we treated humanized mice that were transfused with infected, primary CD4+ T Cells. To measure removal of HIV latent reservoirs, CD4+ T cells were isolated from people living with HIV (PLWH) under suppressive antiretroviral therapy. These cells were plated in limiting dilution for a quantitative viral outgrowth assay to measure reductions in reservoirs upon treatment.



Graphical Abstract

Results: High micromolar concentrations are needed for efficient killing of HIV-1 infected cells due to NNRTIs' binding affinity for human serum proteins. Treatment of HIV infected cells with VbP in combination with NNRTIs enhances killing. VbP alone also induces targeted killing of infected cells. Combination treatment was able to restore NNRTI efficacy in the presence of human serum and can partially overcome NNRTI resistance. We also show that VbP enhances clearance of HIV-1 infected cells in humanized mice and HIV-1 reservoirs from PLWH.

Conclusions: DPP9 inhibition can sensitize the CARD8 inflammasome which ameliorates potential barriers to NNRTI efficacy. We show that this combination strategy is an effective treatment for the in vivo elimination of HIV-1 infected cells in humanized mice and ex vivo in HIV-1 reservoirs isolated from PLWH. This work offers promise for utilizing the CARD8 inflammasome pathway for an HIV cure strategy by modulating CARD8 regulation.

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PP 5.4 – 00184

Optimization of Smac Mimetics as HIV-1 Latency Reversing Agents

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Background: Shock & kill approaches seek to purge the latent HIV reservoir by treating patients with therapeutics that activate latently infected cells and lead to their subsequent elimination. However, at this time it is far from clear what type of treatments would make up an effective shock therapy due to adverse effects or a lack of efficacy of most latency reversing agents (LRA). We have previously identified molecules belonging to the class of Smac mimetics as novel LRAs that reverse HIV latency through activation of the non-canonical NF-kB pathway. Importantly, we developed the compound Ciapavir that exhibited greater potency as LRA than clinically tested Smac mimetics. We now report the optimization of novel Smac mimetic compounds for improved HIV-1 latency reversal in vivo.

Methods: Correlating the biochemical and pharmacological properties of a diverse set of existing Smac mimetic compounds with their activities has allowed us to develop novel molecules in this class that exhibit significantly greater potency and efficacy as LRA. Structure-function relationship assessment guided series of iterative medicinal chemistry development to optimize the pharmacological properties of the compounds for HIV-1 latency reversal. The suitability of these molecules for shock & kill

treatment regimens is being evaluated in in vivo safety and efficacy studies.

Results: A BLT humanized mouse model provided proof-of concept for latency reversal following treatment with Ciapavir and allowed us to developed novel Smac mimetics with optimized pharmacological properties. Beyond superior bioavailability and an absence of observed toxicity, in vitro and in vivo studies in mice revealed differences in the molecules' pharmacokinetic properties. Importantly, the novel Smac mimetics display a range of different activation kinetics, encompassing short-acting and long-acting molecules that allow for the careful design of safe and efficacious multiple-dosing regimens.

Conclusions: Smac mimetics as a molecular class show promising in vivo bioavailability and efficacy. However, recent studies indicate the need for multiple-dosing regimens to efficiently target the latent reservoir. Our development of molecules belonging to the de-risked class of Smac mimetics yielded promising candidates for optimized multiple-dosing regimens as part of shock & kill strategies with the goal of eliminating the latent HIV reservoir.

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PP 5.5 – 00026

Targeting Tat/TAR interactions with the superelongation complex for the development of novel treatments for HIV/AIDS

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Background/Aims: A promising approach to achieve a functional cure for AIDS is to block viral transcription and lock the cellular HIV reservoir in a "deep"-latent state—the so-called "block-and-lock" strategy. This strategy has met some initial success with a natural compound, didehydro-cortistatin (dCA), that targets the viral protein Tat, a central regulator of HIV latency and transcription. However, dCA is very expensive to produce and efforts for a cost-effective synthesis pathway and cheaper analogs have not succeeded. *Our goal is to discover novel inhibitors of Tat-dependent HIV transcriptio*n.

Tat hijacks the host superelongation complex (SEC), containing CDK9, Cyclin T1, AFF1/4, and other transcription factors, and recruits it to the viral promoter by binding to the nascent TAR RNA forming at the 5′ end of all HIV transcripts. In recent years, several X-ray structures of the Tat complex with SEC and TAR revealed the structural basis for the regulatory function of Tat in HIV transcription, providing a high-resolution description of a new target for anti-HIV drug discovery.

Methods: We established a robust high-throughput screening assay (HTS) based on homogenous time resolved FRET methodology (HTRF) to search for inhibitors of TAR binding to Tat-SEC. In addition, we established a counter assay to eliminate false positives from the HTS approach.

Results: The first pilot screen with a library of 3800 FDA approved compounds identified 214 compounds, resulting in a 0.05% initial hit rate and Z'-values >0.7. We are in the process of expanding HTS experiments to include much larger compound libraries, and we established a cell-based transcription assay to further characterize promising hits from the in vitro HTS screening.

Conclusion: We are taking advantage of the structural information on Tat-dependent HIV-transcription by performing a systematic search for small-molecule inhibitors of Tat or TAR binding to the host superelongation complex. We expect that some lead compounds from such a novel approach may act as silencing promoting agents and can eventually be parlayed into a block-and-lock strategy to cure AIDS.

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PP 5.6 – 00183

Combination of traditional medicine product (SDK-2) with TNF-α synergistically reactivate latent HIV-1 subtype C in vitro

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Background/Aims: Persistence of latent HIV in resting CD4+ T cells is the principal barrier to cure development. One strategy currently being pursued to eliminate latently infected cells is to stimulate virus production from latency, which has showed limited success in clinical trials. Therefore, new, and improved latency reversing agents (LRAs) are necessary to reactivate latent viral reservoirs, facilitate HIV-1 eradication and cure development. In this study, we undertook to investigate the effect of a plant-based traditional medicine product Nkabinde latent HIV-1 infection.

Methods: A half maximal inhibitory concentration (IC50) for a four-plant based mixture (Mixture) and single plant product (SDK-2) was determined using the ATP assay. In vitro antiviral activity of the Mixture and SDK-2 was assessed against HIV-1 subtype B (NL4.3 and YU2) and subtype C (CM070P.1 and CM019P.1.2) in TZM-bl cells using the luciferase assay. Next, we evaluated the HIV latency reversal potential of the Mixture and SDK-2 alone or in combination with LRAs using HIV-1 subtype B and HIV-1C based latency model, JLAT-B and JLAT-C respectively.

Results: The Mixture extract inhibited NL4.3 (88%), CM070P.1 (92%), CM019P.1.2 (80%) and YU2 (76%) replication at the IC50 of 325 μg/ml while SDK-2 (106 μg/ml) exhibited 64%, 89%, 34% and 37% inhibition of CM070P.1, CM019P.1.2, NL4.3 and YU2 respectively. The Mixture exhibited less than 1% of the reactivation potential. Interestingly, our data show that SDK-2 in combination of TNF-α exhibit a synergistic effect with TNF-a resulting in 65.4% reactivation of latent HIV-1 subtype C.

Conclusion: Taken together, our data show that while both the Mixture and SDK-2 exhibit both inhibitory and stimulatory effect on HIV-1 infection, SDK-2 exhibit a pronounced reactivation potential in combination with TNF-α. Future experiments will assess the reactivation of SDK-2 in primary CD4+ T cells obtained from people living with HIV who are on suppressive antiretroviral therapy.

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Session 6: Cell & Gene Therapies

OP 6.1

It's a mountain not a hill: Progress made in realizing AAV-delivered inhibitors for an HIV cure

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Antiretroviral drug therapies (ART) suppress plasma HIV viremia in infected individuals to undetectable levels and their usage has prolonged the lifespan for people living with HIV (PLWH). However, only 26 million people have access to these drugs. Additionally, ART does not have the ability to eliminate the viral reservoir and thus, stopping ART results in viral rebound. In contrast, broadly neutralizing antibodies (bNAbs) can kill infected cells through antibody-dependent mechanisms and could be useful for HIV cure strategies. Our work has focused on demonstrating that bNAbs expressed through adeno-associated virus (AAV) vectors can suppress HIV infection like ART and also reduce the viral reservoir. In pilot studies, we have shown that AAV-delivered eCD4-Ig, an antibody-like HIV entry inhibitor, can suppress SHIV and SIV viremia despite low levels of expression. However, some animals that received AAV-eCD4-Ig vectors resulted in uncontrolled viral rebound. To improve the consistency of our gene therapy strategy in rhesus macaques, our latest studies have identified key factors that improve expression in both mice and rhesus macaque models such as using the AAV9 capsid for intramuscular inoculation and using a P2A peptide to express bNAbs. These step-by-step improvements may ultimately lead to realizing a "one-shot" cure strategy for PLWH.

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OP 6.2 – 00195

Targeted genome engineering of human t cells in vivo for HIV cure

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Background: The only successful HIV cure strategy, thus far, has been transplantation of naturally HIV resistant CCR5Δ32 stem cells. This approach is not currently scalable nor feasible or desirable in the general population of persons living with HIV (PLWH). In vivo genome engineering of hematopoietic stem and T cells for HIV resistance may address this issue.

Methods: As a key step toward clinical application, we have generated DNA-free virus-like particles (VLP) surface-decorated with an antibody to human CD7 (aCD7) to allow transient and "scar-less" delivery of packaged CRISPR-Cas9 ribonucleoprotein complexes (RNPs) to human T cells and monocytes. aCD7:VLP packaged with Cas9/RNPs were tested in humanized mouse models of HIV-1 infection for the targeted editing of the CCR5 gene in human T cells after intravenous (IV) administration.

Results: IV administration of R5-tropic HIV-infected, ART-suppressed humanized mice with aCD7:VLP encapsulating Cas9/RNP targeting CCR5 resulted in the specific targeting of human T cells and editing of the CCR5 gene and abrogating surface CCR5 expression at efficiencies of >50%. CCR5-edited CD4 T cells selectively expanded after ART interruption, leading to stabilization of peripheral blood CD4 T cell levels, control of plasma viral loads, and ART-free remission with no outgrowth of infectious HIV-1 from splenocytes. ART-free control of viral loads was also achieved in HIV-1-positive donor cell-xenotransplanted humanized mice demonstrating potential applicability as a therapeutic strategy in ART-suppressed PLWH.

Conclusions: The approach we report here represents an important advance to the traditional use of viral vectors in gene therapy in that it obviates the need for isolation and ex-vivo transduction of hematopoietic cells. We expect our work to be a significant advance towards achieving ART-free remission and of compelling interest to the fields of clinical gene-therapy for HIV-1 and other diseases.

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OP 6.3 – 00150

Delivery and long-term expression of CCR5-blocking monoclonal antibody Leronlimab with AAV for ART-free remission from SHIV viremia

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Background: CCR5 blockade represents a scalable non-transplantation approach for long-term ART-free HIV remission. Here, we tested if AAV vectors could induce long-term expression of CCR5-blocking monoclonal antibody Leronlimab in a SHIV-infected rhesus macaque (RM) and Mauritian cynomolgus macaque (MCM).

Methods: One RM received AAV9 encoding macaque Fc Leronlimab with stabilizing, silencing, and half-life extending mutations (AAV9-MacLSLeron) with no immune suppression. One MCM received AAV9 encoding human Fc Leronlimab (AAV9-HuLeron), and to limit immune activation, received three doses of dexamethasone (−12, −1, and 5 hours post-AAV) and daily tacrolimus (days −8 to 28 post-AAV).

Results: The AAV9-MacLSLeron-treated RM reached 100% CCR5 receptor occupancy (RO) on blood CD4+ T cells within 1 week and plasma Leronlimab was detected (>1 ug/ml) within 2 weeks of administration. Antidrug antibodies (ADA) developed 4 weeks post-AAV, but then resolved by 6 weeks post-AAV. Consequently, SHIV viremia became undetectable shortly after resolution of ADA at 9 weeks post-AAV. The AAV9-HuLeron-treated MCM achieved 100% CCR5 RO on blood CD4+ T-cells within 2 weeks and possessed detectable plasma Leronlimab (>1 ug/mL) within 3 weeks without ADA, with CCR5 RO and plasma Leronlimab maintained through 33 weeks post-AAV. Mesenteric lymph node and spleen CD4+ T-cells from week 13 post-AAV exhibited >98% RO. SHIV viremia became undetectable within 4 weeks post-AAV and remained undetectable through 33 weeks post-AAV with the exception of 3 blips of plasma viremia, which coincided with small transient dips in blood CD4+ T-cell CCR5 RO.

Conclusions: While further investigation is needed to develop AAV vectors and/or regimens that reduce the incidence of ADA, these data demonstrate the potential of AAV vectors for sustained antibody-based CCR5 blockade as a gene therapy approach for long-term ART-free HIV remission.

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OP 6.4 – 00096

High-efficiency CRISPR/Cas9-mediated disruption of ccr5 in human hematopoietic stem progenitor cells generates HIV-refractory immune systems

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Background: Hematopoietic stem cell transplant (HSCT) with CCR5D32/D32 stem cells has been attributed to long-term remission of HIV infection in three patients ("Berlin," "London," and "Dusseldorf") who received allogeneic HSCT for co-occurring malignancies. However, the scarcity of recipient HLA-matched, ccr5D32 homozygous stem cell donors is one of the most significant hurdles to widespread adoption of HSCT therapy for HIV infection.

Methods: A combination of in silico prediction software and in vitro screening methods were used to develop guide RNAs (gRNAs) capable of efficiently editing the human ccr5 gene. off-target gene regions containing sites with <4 base pair mismatches were amplified and deep sequenced to analyze indel frequency formation. Using these optimized guides, we describe the application of a scalable CRISPR-Cas9/RNP platform to achieve high frequency ccr5 editing in adult HSCs (85–95% ccr5 disruption). Ccr5-edited HSCs were engrafted into 6–8 week-old female NSG mice to evaluate hematopoiesis and to generate an in vivo HIV challenge model.

Results: After HSCT transplant into xenograft mice, ccr5-edited HSCs displayed slightly delayed but otherwise normal hematopoiesis resulting in immune reconstitution with typical frequencies of human monocytes, B cells, and T cells. High frequency of ccr5 editing was detected in the descendant myeloid (84–94% ccr5 disruption) and lymphoid lineages (95–97% ccr5 disruption), and the frequency of T cells expressing CCR5 on the cell surface was reduced >100-fold. Importantly, mice engrafted with ccr5-edited HSCs were refractory to multiple challenges with CCR5-tropic HIV, including a challenge dose 5 times higher than the dose which infected all control mice. We also establish a threshold of 72% ccr5 ablation in HSCs as being required to significantly resist HIV infection upon differentiation.

Conclusions: This study demonstrates the feasibility of developing a CRISPR-Cas9/RNP approach to efficiently edit ccr5 in human hematopoietic stem cells and supports the continued development of an autologous HSCT-based cure for HIV infection.

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OP 6.5 – 00164

Nanobody-engineered AAV vectors for CD4-targeted gene therapy

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Background: HIV cure approaches involving in vivo gene therapy have the potential to target prominent tissue-resident HIV reservoirs. Virus-based vectors are most common in clinical studies, with Adeno-associated Virus (AAV) being the vector of choice. Effectiveness of in vivo gene therapy is largely correlated with high vector doses, but must be balanced to avert unwanted effects including immune responses and transgene activity in non-target cells. Modulating receptor binding specificities of therapeutic vectors increases on-target effectivity while reducing off-target effects. So far, no robust viral gene therapy vector specific for CD4-positive HIV target cells has been described. We here show that coupling of AAV capsid surfaces with CD4-specific nanobodies mediates retargeting of AAVs to CD4-positive cells. These CD4-AAVs could provide an invaluable vector for anti-HIV therapeutic approaches.

Methods: We used structure-based rational design to genetically fuse CD4-specific nanobodies into AAV capsid proteins VP1 and VP2. We examined optimal nanobody/insertion-site combination for targeted reporter transgene delivery in vitro and in vivo. We applied our engineering platform to multiple AAV serotypes, proving system versatility. Electron microscopy (EM) and biochemistry analyses were used to investigate particle characteristics.

Results: Functional competition assays with CD4-AAV vectors revealed highly specific transduction of CD4-positive cells in vitro. Isolated human primary CD4-positive T cells from multiple donors are effectively transduced compared to wild-type AAV serotypes. CD4-positive cells were transduced with 5-fold higher specificity compared to CD4-negative cells in vitro. EM and biochemical characterization of CD4-AAV particles revealed overall beneficial features: regular morphology, increased capsid stability, nanobody presentation on the capsid outer shell, exceeding titers and unaffected genome packaging compared to wild-type AAVs.

Conclusions: We provide proof-of-concept for a novel AAV-based vector platform with high specificity towards CD4-positive cells. These nanobody-engineered AAVs further preserve all beneficial AAV vector characteristic. Adaptation to other serotypes and exchange of nanobodies is straightforward. We propose CD4-AAV vectors as valuable tool for safe and efficient anti-HIV in vivo gene therapy approaches.

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OP 6.6 – 00134

Viral Suppression in SHIV-infected Rhesus Macaques following AAV-mediated Delivery of Closer-to-germline Monoclonal Antibodies

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Background: We have three SHIV-infected monkeys which, after receiving AAVs encoding a cocktail of neutralizing anti-HIV antibodies during the chronic phase of infection, have shown suppressed viral loads for years and appear to have been functionally cured. Unfortunately, our attempts to create more such functional cures have been severely hampered by the generation of anti-drug antibody responses (ADAs) to the AAV-delivered antibodies. These ADA responses have also been a problem in human phase I clinical trials of AAV delivery of bNAbs. Due to years of affinity maturation, most bNAbs exhibit unusually high levels of somatic hypermutation and accumulate uncommon features that can be seen as 'non-self' by the recipient's immune system. Consequently, unwanted ADA responses can be raised against the AAV-delivered bNAbs, compromising their efficacy by reducing their concentration and functionality. To overcome this critical issue, we attempted delivery of less mutated antibodies, i.e. antibodies closer to germline.

Methods: Four Indian-origin rhesus macaques were experimentally infected with SHIV-AD8. At week 14 post-SHIV infection these monkeys received recombinant AAVs expressing three broadly neutralizing antibodies (DH270, PCIN63 and DH511) that were naturally closer to germline than those we had used previously. In vivo circulating antibody and ADA levels were measured over time by ELISA.

Results: High levels (22–327 μg/mL) of two AAV-delivered antibodies were obtained in three of the four macaques through the 26 weeks of measurements. Sustained viral load suppression was achieved in one of those three monkeys. A second one was successfully suppressed for 20 weeks and viral rebound was detected in the last two measurements. The third monkey showed only transient effects on viral load levels. Escape mutant virus is suspected in these two last monkeys. The fourth monkey had low antibody levels due to ADAs and little or no virologic suppression. Overall ADA levels correlated well with the AAV-delivered antibody levels: the lower the ADAs, the higher the antibody levels.

Conclusions: Our data indicate that the use of closer-to-germline bNAbs may be a viable strategy for overcoming ADAs following gene therapy with AAV-bNAb vectors but they also highlight the difficulties associated with achieving long-term suppression.

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OP 6.7 – 00044

Long-term ART-free SIV Remission Following Allogeneic Hematopoietic Cell Transplantation in Mauritian Cynomolgus Macaques

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

Methods: Grafts collected from MHC-matched, SIV-naïve donors were transplanted into four SIV+, ART-suppressed recipients following reduced intensity conditioning (RIC). Donor chimerism was measured by sequencing SNPs. Total and intact SIV DNA levels were measured by SIVgag qPCR and intact proviral DNA assays, respectively. Recipients were maintained on ART until analytic treatment interruption (ATI) at 700–900 days post-HCT.

Results: Following RIC and HCT, lymph node CD4+ T cell-associated SIV DNA levels decreased ~10-fold within 30 days in all four recipients. Recipient 1 reached 100% donor chimerism without further intervention. Recipients 2, 3, and 4 presented with mixed T cell chimerism and received donor lymphocyte infusions (DLIs), which successfully induced full donor chimerism in recipients 3 and 4, but not recipient 2. Graft-versus-host disease (GVHD) manifested in recipients 3 and 4, but not recipients 1 or 2. In all recipients and across tissue reservoirs, we observed a strong inverse correlation between cell-associated SIV DNA and donor chimerism in CD4+ T cells. Prior to ATI, intact SIV was undetectable in blood, lymph node, spleen, and bone marrow in GVHD-experiencing recipients 3 and 4, but persisted in recipients 1 and 2. Upon ATI, recipient 2 rebounded with SIV viremia within 11 days while recipients 1, 3, and 4 remained aviremic for >17 weeks. Recipient 1 experienced SIV rebound at week 18, while GVHD-experiencing recipients 3 and 4 remain in SIV remission >2 years post-ATI despite CD8-depletion. SIV Env-binding antibody titers fell below 15 ug/mL in all four recipients post-HCT, increased above 100 ug/mL in recipients 1 and 2 upon rebound of SIV plasma viremia post-ATI, but remain below 15 ug/mL in aviremic recipients 3 and 4 post-ATI.

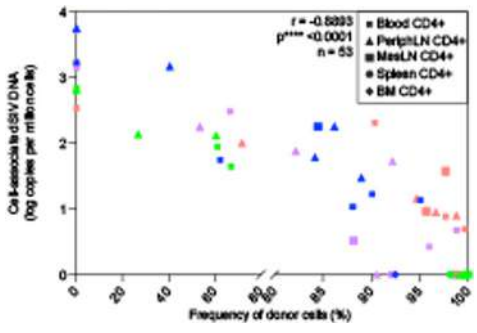

Figure. Paired donor chimerism and SIV DNA post-HSCT.

Conclusions: These data demonstrate that alloHCT-mediated viral reservoir clearance occurs concomitantly with full CD4 + T cell donor chimerism and is associated with GVHD, suggesting that allogeneic immunity drives viral reservoir clearance in alloHCT-mediated HIV cure.

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OP 6.8 – 00102

In vivo evolution of env in SHIV-AD8-infected rhesus macaques after AAV-eCD4-Ig therapy

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Background: eCD4-Ig is a potent HIV entry inhibitor consisting of the ectodomain of CD4, an IgG Fc portion, and a short tyrosine-sulfated peptide that resembles the tyrosine-sulfated regions of all primate lentivirus coreceptors. eCD4-Ig mimics the engagement of both CD4 and CCR5 with the HIV Env, a property which imbues it with remarkable neutralization breadth. However, env is exceptionally genetically malleable and can evolve to escape a wide variety of entry inhibitors. Here we document the evolution of partial eCD4-Ig resistance in SHIV-AD8-infected rhesus macaques (RMs) treated with AAV encoding eCD4-Ig.

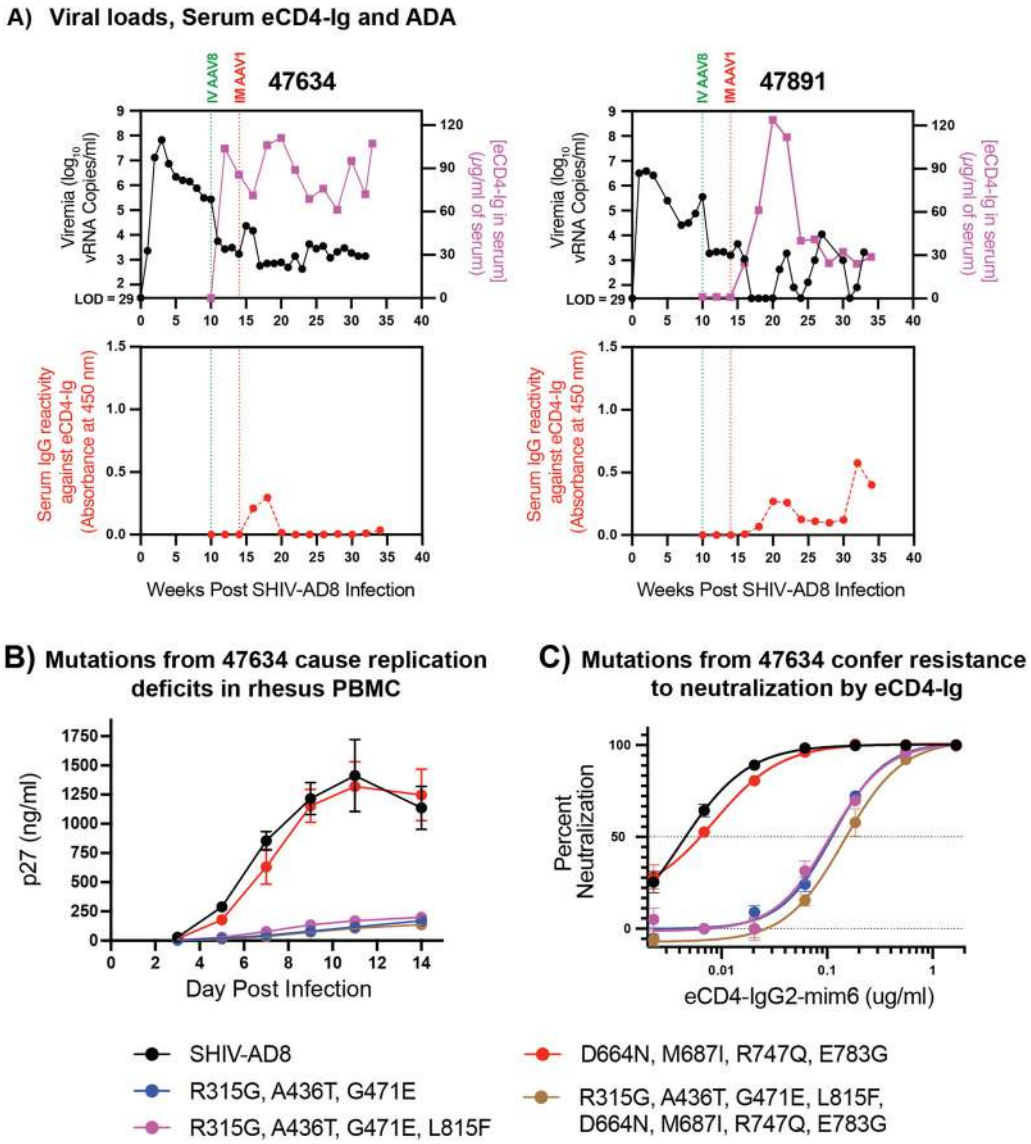

Figure 1 (abstract: OP 6.8–00102)

Methods: Six RMs were subjected to repeated, escalating dose, oral inoculations of SHIV-AD8 until they became infected. At 10 and 14 weeks after the onset of viremia, five of the six macaques were administered AAV encoding eCD4-Ig. Viral load, serum eCD4-Ig, and anti-drug antibodies (ADA) were monitored for the duration of the study. Longitudinal env sequencing was performed for two animals that experienced consistent serum levels of eCD4-Ig. Mutations recovered from one animal with a striking phenotype were cloned and further characterized.

Results: Two of the five treated RMs developed persistent levels of eCD4-Ig in serum but experienced only partial or transient reductions in viremia. Six mutations became fixed at 100% frequency in plasma, two of which evolved independently in both animals. In one of the two RMs (47 634), setpoint viremia plateaued at 1000 vRNA copies/ml, despite concomitant serum concentrations of eCD4-Ig in the 70–100 μg/ml range. Three mutations recovered from this animal (R315G, A436 T, G471E) were sufficient to confer substantial resistance to eCD4-Ig-mediated neutralization on the parental SHIV-AD8 Env, accompanied by a marked cost to replicative fitness in activated rhesus PBMC.

Conclusions: R315G, A436 T, and G471E appear to confer eCD4-Ig resistance in part by taking advantage of a single amino acid difference between rhesus CD4 and eCD4-Ig (I39N) and in part by modulating CCR5 engagement, potentially allowing for use of alternate coreceptors. Taken together, our results shed light on the evolutionary pathways that remain open to env when it is confronted with a broadly neutralizing mimic of its major natural entry receptors.

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PP 6.1 – 00005

Single cell quantification of hiv-1 and lentiviral vector in gene therapy studies

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Background: Lentiviral-based vector therapies, which include many CAR-T and gene-modified cell therapies, are at the forefront of HIV-1 cure research. However, sequence homology between lentiviral vectors and HIV-1 creates challenges with quantifying cellular HIV burden and lentiviral DNA. As a result, we developed a novel single-cell in-droplet (scd)PCR method to co-quantify genomic HIV-1 DNA and lentiviral vector DNA (from CAR-T and gene-modified autologous stem cell transplant (SCT)) with single-cell resolution. This approach provides a high-throughput platform to determine if gene-modified cells can become infected *in vivo*, a critical unknown question.

Methods: We adapted a multiplexed scdPCR method to co-quantify genomic HIV-1 DNA and MNDU3 promoter, a common component of lentiviral gene delivery vectors. In addition, we screened over 30 existing HIV-1 DNA quantitative assays to determine the optimal method to quantify HIV DNA in cells from human studies.

Results: First, the figure shows that commonly used lentiviral vectors directly cross-react with the HIV-1 LTR, LTR-Gag junction/Psi, and Env regions. Second, we observed that the intact proviral DNA assay (IPDA), co-targeting Psi/Env, quantified nearly 100% of lentiviral vector DNA. Third, of over 30 screened HIV-1 qPCR assays, we identified only one, targeting the downstream Gag region, that did not have significant cross-amplification with lentiviral vector DNA. Fourth, we successfully duplexed the HIV-1 and MNDU3 DNA assays for droplet-digital (dd)PCR with minimal cross-amplification using both physiological and super-physiological levels of HIV-1 and vector DNA in bulk cell lysates from combinations of 293 T cells transfected with: (a) HIV-1 plasmid, (b) 1TAX (lentiviral vector), (c) HIV-1 and 1TAX, (d) non-transfected. Finally, we have successfully applied the duplexed ddPCR assay in our scdPCR platform for use with individually-encapsulated, transfected 293 T cells. The multiplexed ddPCR assay is being implemented on participant-derived cells from a multiple gene-modification study in autologous SCT (AMC097) and duoCAR-T cell study in people with HIV.

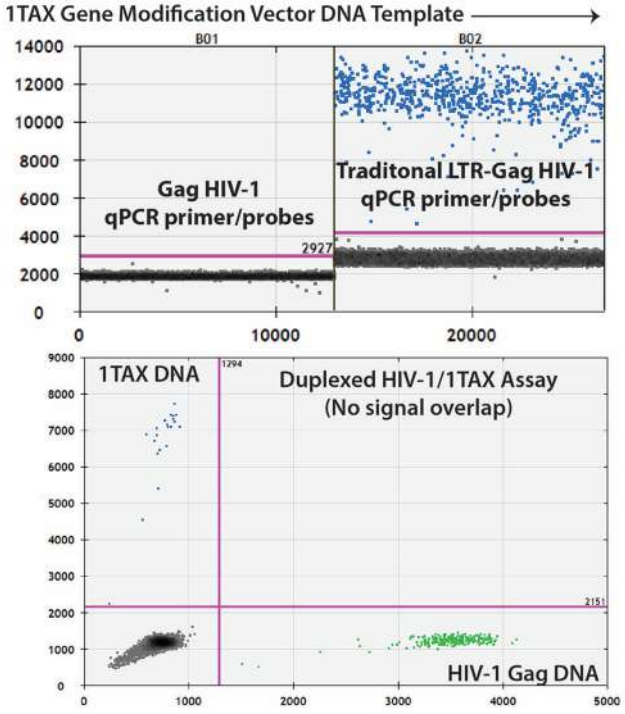

Figure. Duplex of MNDU3 Promoter and HIV-1 Gag Region.

Conclusions: The multiplexed scdPCR assay can reliably quantify both HIV-1 DNA and lentiviral vector DNA in cell lysates and in individually-encapsulated cells. This method also allows in-depth characterization of residual HIV-1 burden and the potential for infected, transduced cells *in vivo/ex vivo* across a range of gene-modification studies.

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PP 6.2 – 00106

CAR/CXCR5 T cells contact HIV vRNA+ cells in HIV-infected humanized DRAGA mice

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Background: HIV-specific chimeric antigen receptor T (CAR T) cells are being developed as a potential approach towards curing HIV infection. During infection, HIV replication is concentrated in B cell follicles, and viral reservoirs such as B cell follicles are a significant barrier to an HIV cure. We developed HIV-specific CAR T cells expressing the follicular homing receptor CXCR5 (CAR/CXCR5 T cells) to target follicular HIV reservoirs. We hypothesized after infusion of CAR/CXCR5 T cells in humanized HIV-infected DRAGA mice, CAR/CXCR5 T cells would accumulate in lymphoid follicles, make direct contact with HIV+ cells, lead to reductions in HIV viral loads, and preserve human CD4 T cells.

Methods: Fourteen female humanized DRAGA mice were included in this study. Twelve mice were infected with 10 000 TCID50 of HIV-1 BaL. Levels of HIV-1 plasma viral loads and CD4 T cells were monitored using qRT-PCR and flow cytometry. Two spleens from uninfected mice were used to produce transduced CAR/CXCR5 T cells and transduced cell products (2×105 cells/gram) were infused in six HIV-infected mice. RNAscope combined with immunohistochemistry was used to visualize locations and quantities of CAR/CXCR5 T cells and HIV vRNA+ cells in lymphoid tissues.

Results: All mice were HIV-1 detectable nbefore infusion of CAR/CXCR5 T cells. High levels of CAR/CXCR5 T cells and HIV vRNA+ cells were detected at 6 days post-infusion in lymphoid tissues. Many CAR/CXCR5 T cells were found in direct contact with HIV vRNA+ cells. However, many CAR/CXCR5 T cells, presumably CD4+ cells, were HIV vRNA+ and likely spreading infection. No differences in HIV plasma viral loads or CD4 T cell counts were observed between control and treated animals.

Conclusions: These studies support the use of the HIV-infected DRAGA mouse model for HIV cure research studies. Using this model, we showed CAR/CXCR5 T cells accumulate in follicle-like structures with HIV vRNA+ cells and come in contact with vRNA+ cells. The simultaneous detection of CAR T cells with high levels of HIV vRNA+ cells indicates the need for HIV-resistant CAR T cells. These preliminary findings demonstrate the HIV-infected DRAGA mouse model is extremely valuable for evaluating HIV cure approaches.

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PP 6.3 – 00178

Targeting the human and macaque CCR5 genes using the CRISPR-SaCas9 gene-editing platform

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Background: C-C Chemokine receptor type 5 (CCR5) plays a key role in HIV infection as a co-receptor for HIV entry into host cells and cell-to-cell spread. The crucial role of CCR5 in HIV infection was confirmed by the discovery of the delta-32 mutation in the coding region of CCR5. People homozygous for this mutation are resistant to HIV infection. Furthermore, CCR5Δ32/Δ32 hematopoietic stem cell transplantation was found to cure HIV-1 disease in three individuals: "a Berlin patient," "a London patient," and most recently, a woman from New York.

Methods: In this study, we examined the potential application of the CRISPR-SaCas9 platform to edit the human and macaque CCR5 genes. First, the set of candidates SaCas9 specific gRNAs targeting coding sequences of CCR5 genes was selected using CRISPOR online platform and then tested in human and simian cell lines and primary human and simian PBMCs. Briefly, cells were electroporated with control or CRISPR-CCR5 ribonucleoprotein complexes, and after 48 h, genomic DNA was extracted and subjected to CCR5-specific PCRs. Next, the CCR5 amplicons were purified from the gel, sequenced, and analyzed using the ICE (Inference of CRISPR edits) analysis tool. The single gRNAs showing the most efficient on-target cleavage activity were then paired, enabling CRISPR-mediated excision of the CCR5 gene. Finally, PCR-genotyping/Sanger sequencing was used to confirm the efficient and specific excision of the CCR5 gene in primary immune cells. In addition, the same technique was used to verify the lack of off-target activity of selected gRNAs in the human and macaque genomes.

Results: The ICE analysis identified two gRNAs having on-target CRISPR InDels frequency scores above 50%, specific for both human and monkey CCR5 genes. After pairing the gRNAs and analyzing them by PCR, we found that some combinations were able to excise a sequence from the CCR5 gene. The sequencing data confirmed the perfect excision/end-joining of CCR5.

Conclusions: These strategies will explore the curative activities of gene editing of cellular receptors to reduce the latent HIV/SIV cell reservoir and delay viral rebound after discontinuation of ART.

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PP 6.4 – 00203

Construct a series of universal gRNAs targeting various regions within HIV

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Background: The persistence of proviral DNA in infected CD4+ leukocytes presents a barrier to HIV-1 treatment. HIV is one of the most genetically diverse pathogens due to its high mutation and recombination rates, large population size, and rapid replication

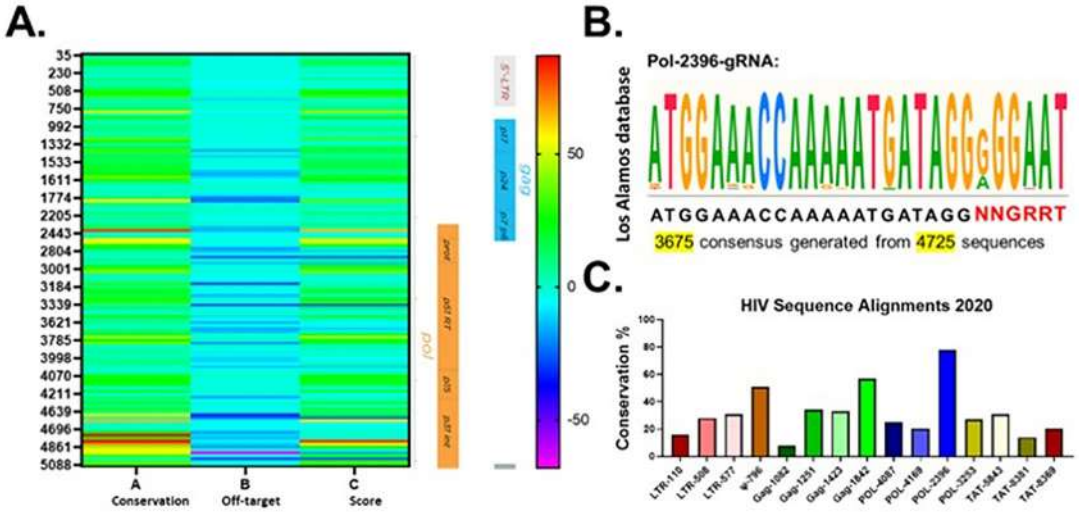

Figure 1 (abstract: PP 6.4–00203) Bioinformatics analysis of gRNA conservation.

rate. Coinfection and superinfection by divergent HIV strains have become more common. While CRISPR-saCas9 can eliminate latent proviral DNA, its efficacy is limited by HIV strain diversity. Thus, it's critical to design a global patient coverage gRNA.

Methods: We initially use pNl43 as a reference strain to list all potential gRNAs that target LTR, Gag, Pol, and Tat. Bioinformatics analysis was performed to check gRNA conservation with 4725 complete sequences from the Los Alamos database. Fifteen potential gRNA were selected, and the efficacy of those gRNAs were affirmed by electroporation with through Lonza electroporation system in Myeloid cells and T cells. Treated cells were evaluated for viral DNA excision spanning target for gRNA by monitoring HIV-1 DNA, protein, and progeny virus levels.

Results: The virus was reduced by up to 93% after single-gRNA CRISPR RNP treatments, respectively. No recorded off-target cleavages were detected. gRNA-LTR-508, gRNA-ψ-796, gRNA-Gag-1842, and gRNA-Pol-2396 as best candidate gRNA due to their high conservation (up to 79%), low off-target, and high efficacy were selected to do single gRNA and combination dual gRNA screen in ex vivo with primary CD4+ T cells. Up to 22% indel mutation was detected with single gRNA treatment and around 50% HIV p24 decrease in dual treatment primary CD4+ T cells.

Conclusions: Our results demonstrated that our gRNAs afford broader antiviral coverage with low off-target, and high efficacy providing a promising step in the direction of eliminating HIV-1 infection through gene editing approaches.

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PP 6.5 – 00205

Utilization of high-throughput assays and deep-learning for selection of CRISPR/Cas9-gRNA pairs used in an HIV-1 cure strategy

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Background: Human immunodeficiency virus type 1 (HIV-1) persistence has been attributed to the latent viral reservoir of integrated proviral DNA in tissues including the peripheral blood, lymphoid tissue, brain, gut, and likely other tissues. An in silico prediction algorithm trained on a dataset of patient-derived LTRs isolated from PBMCs led to the discovery of broad-spectrum selected molecular guide RNA (gRNA) targets (SMRT) gRNAs.

Methods: In silico computational pipelines were designed to examine HIV genetic variation in conjunction with cas9 position weight-matrices to identify broad-spectrum gRNAs. In vitro gene-editing in various cell lines (TZM-bl, P4R5, and J-Lat cells) were analyzed post-transfection or transduction for CRISPR edits. High-throughput gRNA assays were designed to identify new gRNAs.

Results: SMRT gRNAs are predicted to cleave 100% of patient-derived LTR samples as well as a dataset of publicly available patient sequences, which would lead to inactivation or excision of the integrated provirus. In vitro study of the SMRT gRNAs revealed high cell viability and cleavage activity through flow cytometry and fluorescent microscopy in TZM-bl, P4R5, and J-Lat cell systems. Sequences of HIV-1 LTR have been obtained from an independent set of patient-derived tissue samples from the NNTC in the brain and spleen with Illumina NextSeq. The

PBMC-derived SMRTs showed >90% predicted efficacy for the brain sequences. Furthermore, the Multiple Lentiviral Expression System (MULE) is currently being used to engineer lentiviral constructs to deliver Sa or Sp Cas9 and targeted gRNAs to cells. These lentiviruses will be produced in a library approach to simultaneously measure the ability of thousands of gRNAs to silence HIV-1 expression.

Conclusions: Accumulation of this data as well as delivery of a library of gRNAs will allow analysis of Sa and Sp Cas9 enzyme efficiency, gRNA specificity – including consideration of the effect of a leading G nucleotide in the protospacer, and on- and off-target cleavage efficacy.

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PP 6.6 – 00208

Construct a series of universal gRNAs targeting various regions within HIV

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[2]*Excision Biotherapeutics, Boston, USA*

Background: The persistence of proviral DNA in infected CD4+ leukocytes presents a barrier to HIV-1 treatment. HIV is one of the most genetically diverse pathogens due to its high mutation and recombination rates, large population size, and rapid replication rate. Coinfection and superinfection by divergent HIV strains have become more common. While CRISPR-saCas9 can eliminate latent proviral DNA, its efficacy is limited by HIV strain diversity. Thus, it's critical to design a global patient coverage gRNA.

Methods: We initially use pNl43 as a reference strain to list all potential gRNAs that target LTR, Gag, Pol, and Tat. Bioinformatics analysis was performed to check gRNA conservation with 4725 complete sequences from the Los Alamos database. Fifteen potential gRNA were selected, and the efficacy of those gRNAs was affirmed by electroporation with through Lonza electroporation system in Myeloid cells and T cells. Treated cells were evaluated for viral DNA excision spanning target for gRNA by monitoring HIV-1 DNA, protein, and progeny virus levels.

Results: The virus was reduced by up to 93% after single-gRNA CRISPR RNP treatments, respectively. No recorded off-target cleavages were detected. gRNA-LTR-508, gRNA-ψ-796, gRNA-Gag-1842, and gRNA-Pol-2396 as best candidate gRNA due to their high conservation (up to 79%), low off-target, and high efficacy were selected to do single gRNA and combination dual gRNA screen in ex vivo with primary CD4+ T cells. Up to 22% indel mutation was detected with single gRNA treatment and around 50% HIV p24 decrease in dual treatment primary CD4+ T cells.

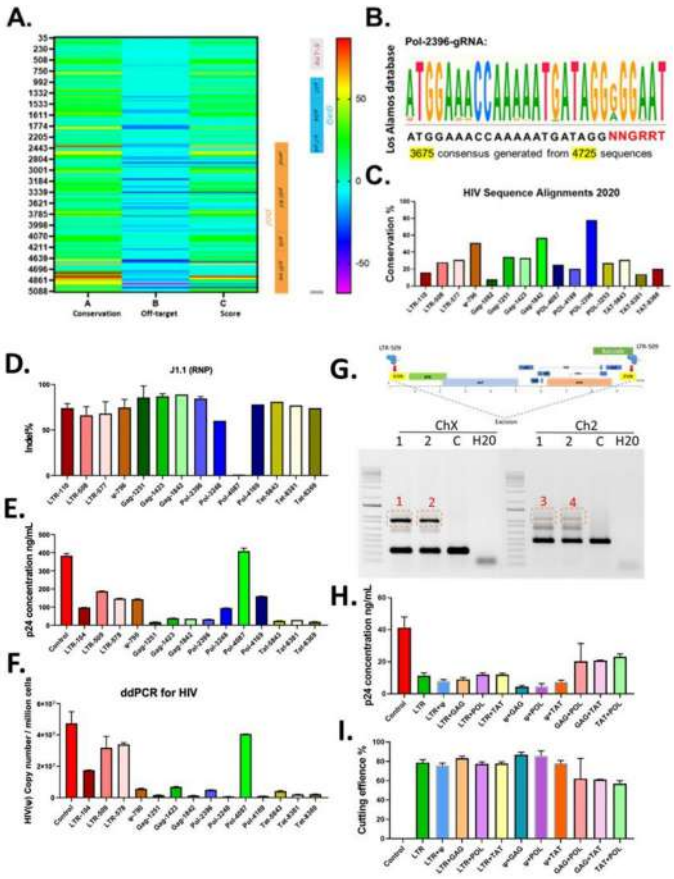

Figure. Single gRNA and combination dual gRNA screen.

Conclusions: Our results demonstrated that our gRNAs afford broader antiviral coverage with low off-target, and high efficacy providing a promising step in the direction of eliminating HIV-1 infection through gene editing approaches.

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Session 7: Human Studies

OP 7.2 – 00035

Impact of 10-1074LS and 3BNC117-LS on viral rebound dynamics following treatment interruption six months after dosing: four cases from the open label arm of the RIO trial

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#These authors contributed equally to this work.

Background: The RIO trial (NCT04319367) is the first randomised trial in treated primary HIV infection evaluating the impact of single dose, combination long-acting broadly neutralizing antibodies (bNAbs) 3BNC117LS and 10-1074LS on viral rebound after antiretroviral treatment (ART) interruption (ATI) compared with placebo. RIO involves an initial blinded stage, followed by an open-label stage in which placebo recipients are offered bNAb infusions with ART and a second ATI, 24 weeks post-infusion. We present clinical outcomes from four participants from the open-label stage of the study.

Methods: RIO opened to recruitment in May 2021 across 8 UK sites. Eligible participants initiated ART in early HIV infection with no evidence of bNAb resistance by in-silico sensitivity testing. HIV viral load (VL) and CD4 count were monitored during ATIs until viral rebound. ART restart criteria were six consecutive VL measurements >1000 or two measurements >100 000 copies/mL. BNAb serum concentrations were measured by validated anti-idiotype ELISA.

Results: The 4 participants were MSM, mean age 39 years (range 26–52). Rapid viral rebound was observed during the first ATI (median 8.5 weeks after ATI, range 4–10). After commencing ART and bNAb administration (dose: 10 mg/kg (10-1074LS) and 30 mg/kg (3BNC117LS)), median time to HIV viral load resuppression (<20 copies/mL) was 4.5 weeks (range 2–8 weeks). The second ATI occurred six months after bNAb dosing. Two participants restarted ART after 26 and 22 weeks. Two participants have not yet met ART-restart criteria after 32 and 8 weeks. The second ATI exhibited lower peak rebound levels compared to the first (median peak viraemia 3.8 log(10) vs 6.0 log(10) copies/ml, $p = 0.0025$). Serum bNAb concentrations peaked >100 mg/ml for both bNAbs, and mean bNAb concentration 6 months after dosing were 46.3 ug/ml (3BNC117LS) and 32.1 ug/ml (10-1074LS) in 3 participants whose data at this timepoint was available.

Conclusions: Single infusion of combination 10-1074LS and 3BNC117LS at ART restart resulted in serum concentrations >10 ug/ml six months after dosing. All 4 participants lost viral control from undetectable nlevels after the second ATI, but with evidence of delayed rebound compared to the first ATI and 2 log lower plasma peak VL observed to date.

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OP 7.3 – 00139

Pre-treatment Interruption Plasma Metabolites and Glycans Correlate with Time to HIV Rebound and Reservoir Size in ACTG A5345

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[4]*Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA;*
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Background: Pre-analytic treatment interruption (ATI) levels of plasma metabolites and glycans were recently suggested as markers of time and probability of HIV rebound upon antiretroviral therapy (ART) cessation. We examined plasma metabolic and glycomic correlates of time-to-viral-rebound (TTVR) and reservoir size in the ACTG-A5345 study.

Methods: Forty-five individuals who initiated ART during chronic (n = 33) or early (n = 12) infection underwent ATI with a median TTVR (≥1000 copies/ml) of 3 weeks. Pre-ATI plasma glycans and metabolites were measured by lectin microarray and mass spectrometry. TTVR was analyzed as a continuous variable in Cox proportional hazards model or binary outcome (rapid rebound defined as TTVR£3 weeks and slow rebound as TTVR>3 weeks) with Mann-Whitney tests. Spearman's correlations were used to examine associations between glycans or metabolites and pre-ATI levels of: (1) plasma and T cell markers of inflammation/immune activation [multiplex arrays and flow]; (2) peripheral CD4+ T cell levels of intact and total HIV DNA [IPDA], HIV RNA [qPCR], and infectious units per million cells [IUPM, viral outgrowth]; and (3) plasma residual viremia [iSCA].

Results: Consistent with previously analyzed cohorts, pre-ATI levels of several glycans correlated with TTVR, including the immuno-modulatory sialylated glycans, which correlated with delayed rebound (Fig. 1A, B; $P < 0.05$), low residual viremia (Fig. 1C; $P = 0.0097$), low HIV DNA and RNA ($P < 0.05$), and low IUPM ($P < 0.05$). High plasma levels of specific metabolites were also associated with TTVR and reservoir size: (1) L-Ergothioneine (anti-oxidative stress metabolite) was associated with delayed rebound (Fig. 1D; $P = 0.016$) and low intact HIV DNA (Fig. 1E; $P = 0.02$); (2) Succinic acid (an immunoregulatory metabolite) associated with rapid rebound ($P < 0.05$) and high HIV DNA and RNA ($P < 0.05$); and (3) tryptophan catabolism (microbiome-related immuno-modulatory pathway) associated with fast rebound (Fig. 1F; $P < 0.05$) and high residual viremia ($P < 0.05$). Glycans and metabolites associated with delayed rebound correlated with low CD8+ T cell activation (%CD38+HLADR+), high %effector CD8+ T cells, low %TIM3+ T cells, and low sCD163 ($P < 0.05$).

Conclusions: Host metabolic and glycomic pathways, with established immuno-modulatory properties, correlated with TTVR and reservoir size in A5345. These pathways warrant further investigation for their potential prognostic and functional significance in determining viral rebound timing during ATI.

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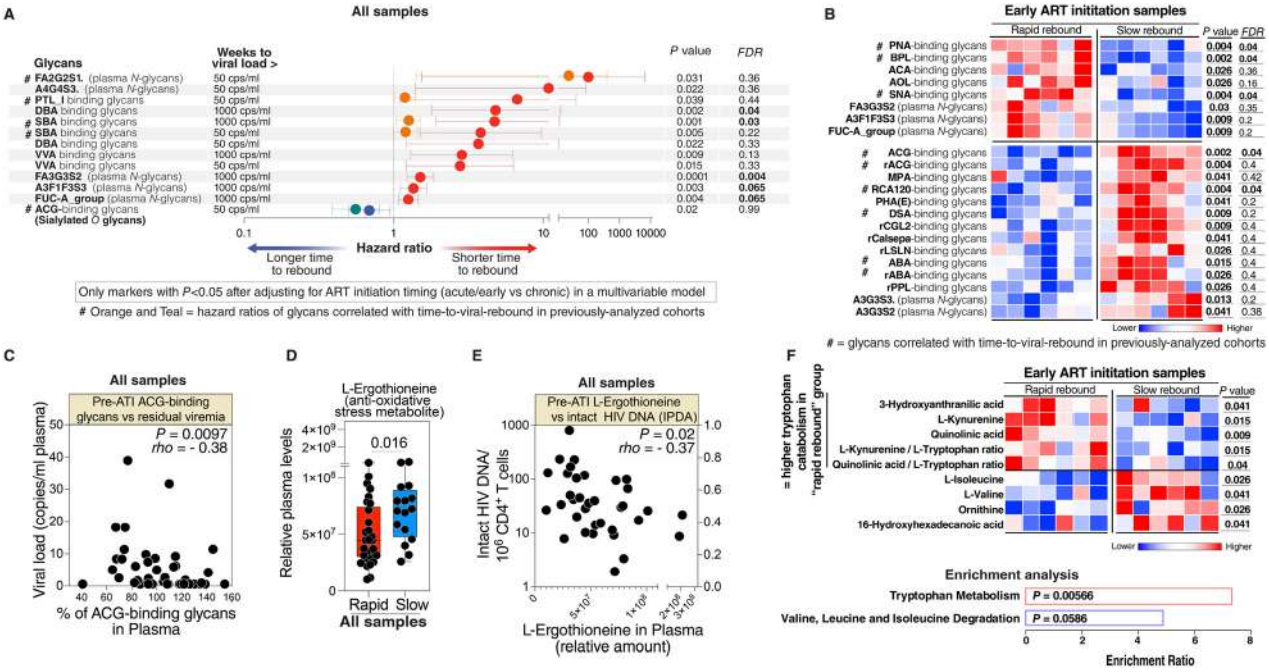

Figure 1 (abstract: OP 7.3–00139)

OP 7.4 – 00012

Series of Jojo. A way to disseminate HIV Cure information in a community language

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Josephine Nabukenya is a young person living with HIV and a founder of Miles of Smiles Foundation a youth led non-government organisation in Uganda that is aimed at creating a smile on every young person. She also supports the social support division to design and implement psychosocial support activities for young people living with HIV at MUJHU Research Collaboration and a published author of Beyond your status, thriving in life in spite of HIV.

Background: Little or no active participation of community people including people living with HIV in the HIV Cure field has been realized for a long time. This can be attributed to the field remaining scientific hence the need to simplify Cure science into a community language that everyone can understand for it to gain support in terms of people living with HIV advocating for Cure research, recruiting research participants and above all, generating policies in favour of the research in low- and middle-income countries. With that background, a platform known as #seriesofjojo was created to simplify HIV Cure information into community language and disseminate it in a youth-friendly animation and illustration way via social media with a primary target of adolescents and young people.

Methods: We selected a team of adolescents and youth peers from 10 health facilities whom we dived deeper into basic HIV Cure information facilitated by the AVAC team.

We identified scientific terms used during HIV Cure science and we simplified them to community lay language.

Since this happened during the COVID-19 tough times, we formulated a WhatsApp group and decided to disseminate the information through illustrations and animated cartoon-like characters.

Back-and-forth communication with the IAS and the AVAC team was observed to ensure that the translated and imagery characters do not lose the original meaning.

Dissemination of the designed illustrations and animated videos through Facebook, Instagram and YouTube targeting adolescents and young people.

Results: Adolescent and youth peers have been empowered with HIV Cure information in a simplified way as well as tools to use as reference when jazzing about HIV Cure with their peers have been made available. Facebook 1331 views and 679 shares; Instagram 719 likes; Youtube 896 views.

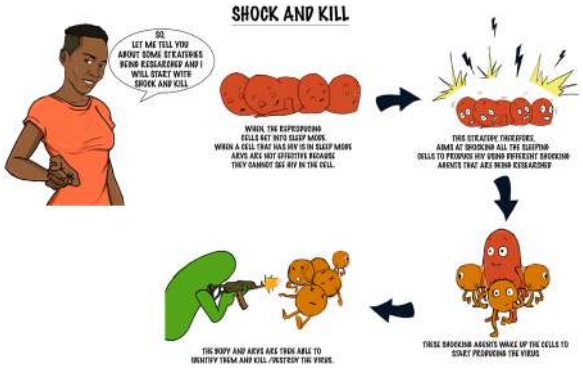

Figure. Shock and Kill illustration.

Conclusions: The quest for HIV Cure information is high, especially among adolescents and young people living with HIV. Creating Series of Jojo platform to simplify the science has enabled them to understand it, demystify myths and misconceptions as well as use the illustrations and animated video as a point of reference when having Cure discussions with their peers.

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PP 7.1 – 00004

Community HIV clinicians' perceptions about HIV cure-related research in the Northwestern USA

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Background: Prior research shows that the decision of people with HIV (PWH) to participate in HIV cure-related research trials is directly affected by HIV clinician input. However, in the context of HIV cure-related research, non-researcher community HIV clinician perspectives remain understudied. We aimed to learn how community HIV clinicians perceive HIV cure research and identify factors they weigh when encouraging or discouraging HIV cure trial participation by their patients.

Methods: We recruited a purposive sample of 12 community HIV clinicians in metro-Seattle, WA to participate in in-depth interviews. We completed 11 interviews via teleconference and received one written response. We used conventional content analysis to analyze the data.

Results: Our findings indicate that community HIV clinicians were generally supportive of patient participation in HIV cure-related clinical trials. Factors affecting support included knowledge of local trials, ease of referral, patient immune function and health stability, study risks and benefits, burden of study requirements, patient characteristics, patient life stability, potential impact on engagement in care, study communication plans, and beliefs that patients should have the autonomy to decide to participate. Participants had concerns about trials requiring treatment delays or interruptions, highlighting the contradictory messages that would be sent regarding the importance of medication adherence. HIV transmission risk during analytical treatment interruptions was also a concern voiced by community clinicians. While they were skeptical of HIV cure discovery in the near term and their knowledge of HIV cure-related research was limited, they were receptive to furthering their knowledge about open HIV cure trials to which they could refer patients. Community clinicians also suggested ways researchers could meaningfully engage them.

Conclusions: It is important for the field of HIV cure research that those leading HIV cure trials increase engagement with non-researcher community clinicians who care for PLWH early in the trial design process. Pre-trial inclusion will facilitate establishment of study and communication protocols with high acceptability, ultimately leading to better enrollment and retention within these essential studies.

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PP 7.2 – 00006

Acceptability of Home-Based Blood Collection Device for Viral Load Testing in HIV Cure Trials with Analytical Treatment Interruptions

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Background: Analytical treatment interruptions (ATIs) are a critical component of HIV cure-directed trials and require weekly or bi-weekly viral load tests that involve frequent venipunctures and significant time commitments. Tasso, Inc. Merck & Co, Inc., Kenilworth, NJ, USA developed a novel viral load home-based blood collection device that could help decrease participant burden during ATIs and promote participation.

Methods: To evaluate the acceptability of the device with people with HIV (PWH), we conducted in-depth interviews via teleconference with participants engaged in two ATI trials with the BEAT-HIV Delaney Collaboratory in Philadelphia, PA. From January 2021–February 2022, we interviewed participants at three time points: (1) within two weeks after participants enrolled in the device study, (2) approximately four weeks after they started to use the device, (3) within two weeks of the ATI ending and ART being re-initiated. We used conventional content analysis to analyze the data.

Results: A total of 17 individuals participated in the study: 15 were cis-gender males, 1 was cis-gender female and 1 transgender woman. Our study showed the high acceptability of the home-based blood collection device. We observed an 87% success rate in drawing blood with a mean of 91.5 devices used per participant. Most PWH found the device convenient, comfortable, easy to use, safe, and painless. Participants viewed the device as a simpler alternative to in-clinic venipuncture blood draws. The main challenge reported was some of the devices did not fill completely. Overall, most participants shared that they had a positive experience with mailing the blood samples and they would consider using the device regularly, even outside of ATIs.

Figure. Summary of ATI Participant Experiences.

Conclusions: Our study illustrates that PWH found the home-based blood collection device for viral load testing valuable within ATI trials. Some participants also expressed a willingness to use the device for non-ATI trials. More research is required before optimizing large-scale implementation of device across ATI trials and to assess the sensitivity of viral load results in context of ATI trials.

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PP 7.3 – 00010

Virion Immunocapture Reveals Low-level Myeloid-derived HIV Expression in Semen under INSTI-based Therapy is Disparate from Circulating Seminal and Blood Proviral Sequences

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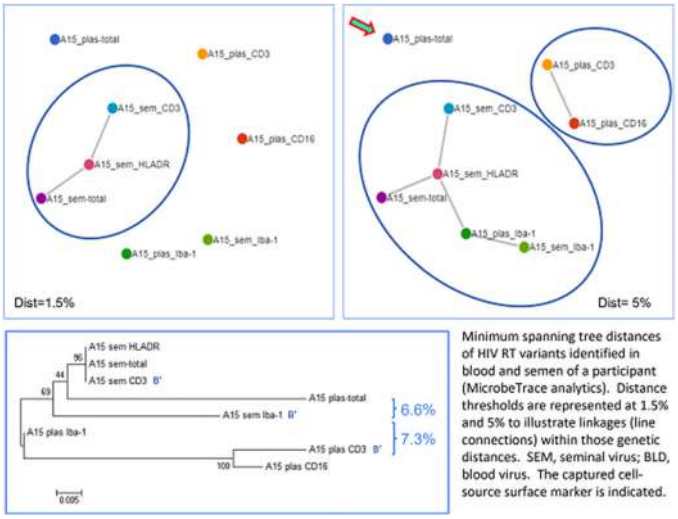
Background: We expand on our earlier reporting of distinct HIV expression between semen and blood in men using INSTI-based regimens to here describe the relatedness of seminal variant genotypes to those of circulating blood and seminal cell proviruses.

Methods: Men living with HIV on INSTI-based regimens for >6 months (n = 15, group A), £6 months (n = 8, group B) or initiating a regimen (N = 1, group C) provided semen, blood, and rectal swabs for virologic examination. Virions were isolated from fresh blood and seminal plasmas by immunocaptures targeting particle-embedded proteins associated with cell source. Amplifiable nucleic acids from captured virion, swabs, and seminal and blood cells were sequenced in pol/RT to assess genetic diversity between specimen types.

Results: Five of 15 group A men had blood virus detected below the limit of quantitation (<30 copies/mL), the remaining were undetectable. Group B individuals had undetectable blood VL except for one at <30 copies/mL. The group C individual VL was 440 000 copies/mL and provided the only swab with amplifiable HIV sequence. HIV captures yielded amplifiable seminal virion RNA from 7, 5 and 1 men in groups A, B and C, respectively. Proviral sequences were obtained from all men except two in group A. Nine men had distinct RT drug-resistant variants segregated by cell-source marker. The immunocaptures found CD3+ (lymphocyte) virus in only three semen samples. Iba-1+ and CD14+ (macrophage-derived) virion in semen and blood was distinct from majority sequences but often appeared as a minority variant link between blood and seminal viruses. The HIV sequence from the group C rectal swab was identical to the blood Iba-1+ variant. Virion genetic distances were as high as 7.4% (23 of 310 nucleotides differed) between compartments over the RT region analyzed (Figure).



Minimum spanning tree distances of HIV RT variants identified in blood and semen of a participant (MicrobeTrace analytics). Distance thresholds are represented at 1.5% and 5% to illustrate linkages (line connections) within those genetic distances. SEM, seminal virus; BLD, blood virus. The captured cell-source surface marker is indicated.

Conclusions: Despite INSTI combination therapy, virus was isolated by immunocapture and often had evidence of multiple myeloid lineage sources with large genetic distances (>2%) both within semen and between semen and blood. Genetic distances were greatest between virion and peripheral cell provirus, pointing to ongoing low-level expression from non-circulating cells. The evidence implies persistent virus expression from tissue-resident myeloid cell sources under potent therapy.

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PP 7.4 – 00016

Acquisition of SARS-CoV-2 infection during an HIV cure study with an ATI period

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Background: Since January 2020 Spain has experienced multiple waves of SARS-CoV-2 (SCV2) infection that have significantly impacted the conduct of clinical trials. of particular concern was whether individuals with HIV infection would be at heightened risk of significant morbidity or mortality associated with acquisition of SCV2 infection. This concern was raised further for individuals with uncontrolled HIV infection. HIV cure research studies require an ATI period when it can be anticipated that participants will experience viremia and provide a unique opportunity to explore the interaction between HIV and SC2 V infections.

Methods: AELIX-003 is a Phase 2, randomized, double blind, placebo-controlled HIV cure study being conducted across eight sites in Spain (NCT04364035). Participants were randomized to receive two HIV vaccines (ChAdOx1.HTI and MVA.HTI) and oral vesatolimod or matched placebos. Two weeks after the final 10th dose of vesatolimod, study participants whose plasma viral load (PVL) was <50 copies/mL plasma and CD4 count >400 cells/mm^3 entered a 24-week ATI period. During the ATI period, participants had weekly HIV PVL and monthly CD4 count quantification. The protocol mandated that individuals who experienced a single PVL of >100 000, eight weekly sequential PVL of >10 000 copies/per mL plasma, or a CD4 count <350 cells/mm^3 were required to restart antiretroviral therapy (ART). SCV2 testing was performed as clinically indicated.

Results: Forty-seven participants entered the ATI period between January 2021 and April 2022. SCV2 vaccination rates were >95%. Eight individuals acquired SCV2 infection during the ATI period. All participants with SCV2 infection had previously been vaccinated against SCV2 infection. There were no hospitalizations and clinical symptoms were G1/G2. No participants were required to restart ART due to loss of virological or immunological control associated with SCV2 infection.

Conclusions: Acquisition of SCV2 during ATI was not associated with significant morbidity or mortality. It is reassuring that no study participants with SCV2 infection crossed the per-protocol virological or immunological thresholds for ART resumption during the ATI period.

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PP 7.5 – 00032

Changes to microglial genome structure and function in the HIV infected brain

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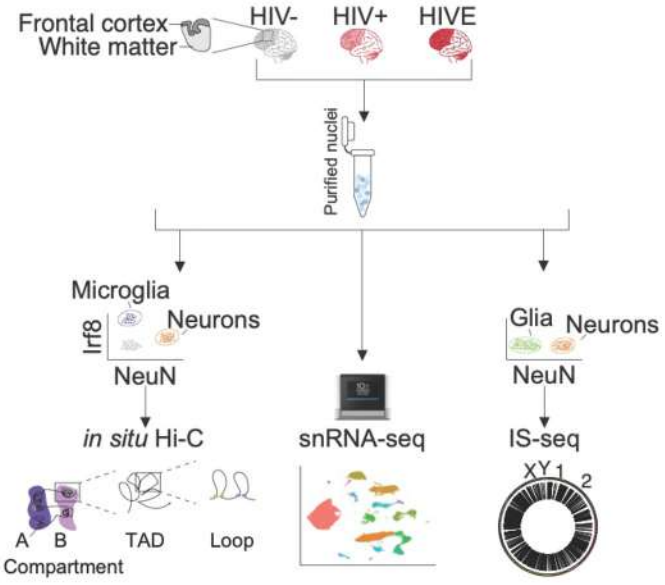
Background: Genomic and transcriptomic exploration of the HIV-infected human brain is critical to understanding and developing treatments for HIV cure strategies. However, to date, genomic studies in HIV i brain have primarily focused on bulk tissue RNA profiling. Herein we describe the first cell-type-specific, integrative genomics studies, including HIV integration site sequencing, chromosomal conformation ('3D genome') mapping, and single nucleus transcription, of frontal lobe tissues from individuals who were HIV-infected with encephalitis (HIVE), HIV infected without encephalitis (HIV+), and HIV-uninfected (HIV−).

Methods: Frontal cortex samples from the Manhattan HIV Brain Bank were processed for 10X Chromium single nucleus RNA-sequencing (snRNA-seq; n = 3 HIV−, n = 3 HIV+ without HIVE [HIV+], and n = 7 HIV+ with HIVE [HIVE]). In situ Hi-C 3D Genome/chromosome conformation mapping was performed on fixed Irf8+ microglial and NeuN+ neuronal nuclei sorted using fluorescence activated nuclei sorting (FANS; n = 2 HIV−, n = 2 HIVE, n = 1 HIV+). Integration site sequencing (IS-seq) was performed on FANS isolated NeuN+ neuronal and NeuN- non-neuronal nuclei for 27 samples (n = 6 HIV−, n = 18 HIV+, n = 7 HIVE). We built an integrative dataset from 79 snRNA-seq, Hi-C, IS-seq, and chromatin ChIP-seq files from postmortem brain, complemented by studies in cell culture.

Results: In both HIV+ and HIVE microglia, we observed decreased expression and repressive compartmentalization of genes involved in homeostatic support functions for the neuronal synapse. In HIVE microglia only, there was also reorganization of open/repressive (A/B) chromosomal compartment structures encompassing 6.4% of the genome, linked to transcriptional activation of interferon (IFN) signaling and cell migratory pathways. Brain IS-seq recovered 1221 integration sites, the vast majority from HIVE non-neuronal nuclei, with distinct genomic patterns as compared to peripheral lymphocyte integration and enrichment for chromosomal domains newly mobilized into a permissive chromatin environment in HIVE microglia. Viral transcription occurred in a subset of highly activated HIVE microglia comprising 0.003% of all HIVE brain nuclei.

Graphical abstract



Conclusions: Our findings point to a disruption of microglia-neuronal interactions in the HIV infected brain and an interrelation of retroviral integration and expression with interferon-associated remodeling of the microglial 3D genome during progression to HIVE.

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PP 7.6 – 00036

Single cell transcriptomics identifies PTMA as a host gene that inhibits HIV during acute infection in vivo

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Background: The viral reservoir during antiretroviral therapy (ART) is the major contributor to HIV persistence and varies between individuals. A better understanding of donors at the acute HIV infection (AHI) timepoint is required to understand HIV infection and reservoir establishment. Single cell technologies can simultaneously capture host and viral transcripts from primary cells during AHI, enabling identification of the cellular actors influencing HIV at this critical timepoint.

Methods: Single-cell transcriptomics was performed on peripheral blood samples from participants at the time of diagnosis of AHI prior to ART initiation, and after 48 weeks of ART when virally suppressed. Host gene expression, TCR diversity and HIV RNA (vRNA) levels from the same individual cells were measured by single cell RNA-sequencing (scRNA-seq). Selected host genes correlating with vRNA were investigated *in vitro*, where they could be overexpressed to assess effects on HIV.

Results: Single cell RNA-seq detected vRNA in CD4+ memory T cells that expressed markers of cytotoxicity and persistence, and also had had higher clonal expansion. Wide sequencing of one participant with the highest viral load and comparisons across cells revealed that vRNA levels associated inversely with expression of the host gene prothymosin alpha (*PTMA*). This association was genome-wide significant and validated in eight additional participants representing different ethnicities and various HIV subtypes. Further, in comparisons across 21 individuals, the frequency of vRNA+ CD4+ memory T cells at the acute timepoint correlated inversely with average *PTMA* expression at the ART timepoint. A direct effect of PTMA on HIV was confirmed by *in vitro* overexpression assays, where increased expression inhibited proviral transcription and infectious virus production in a dose-dependent manner.

Conclusions: HIV can be detected in primary cells from AHI using scRNA-seq. Through unbiased transcriptome analyses in single cells, we identified *PTMA* as the host gene most significantly inversely correlated with vRNA levels. Inter-individual variation further suggested *PTMA* expression inhibits HIV *in vivo* and this was supported by *in vitro* functional data. These results suggest *PTMA* is a novel host restriction factor, of importance given its identification by unbiased transcriptome screening of host cells from primary infection.

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PP 7.7 – 00055

Temporary increase in circulating replication-competent latent HIV-infected resting CD4+ T cells after switch to a Dolutegravir-based antiretroviral regiment

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Background: The primary barrier to an HIV cure is the presence of a latent viral reservoir (LVR) made up primarily of latently infected resting CD4+ (rCD4) T-cells. Studies in the USA have shown that the LVR decays slowly (half-life = 3.8 years), but it is unclear what this decay rate is in Africa.

Methods: This study examined longitudinal changes in the LVR of ART-suppressed Ugandans living with HIV (n = 69) from 2015 to 2020 by measuring infectious units per million rCD4 T-cells (IUPM) derived from quantitative viral outgrowth assays (QVOA). In 2019, Uganda instituted a nationwide rollout of Dolutegravir (DTG)-based ART replacing previous NNRTI-based regimens. Data were analyzed using two versions of a novel Bayesian model that predicted the decay rate of the LVR versus time on ART as a single, linear rate (model A) or allowing for an inflection at time of DTG switch (model B). The models also examined the role of sex and menopause. Outgrowth viruses were deep-sequenced to examine for possible ongoing viral evolution.

Results: IUPM data from a median of four time points per person (range = 2–5, n = 235) were included in the analyses. Model A estimated the slope of LVR change in the population as a non-significant positive increase, suggesting no decay of the LVR. Interestingly, 18% of patients who switched to DTG (11/61) experienced a significant temporary increase in IUPM 6–12 months post-switch (Figure 1). In addition, model B estimated a significant decay pre-DTG switch with a half-life of 7.7 years (IQR = 5.4–12.9), but a significant positive slope post-DTG switch leading to a doubling-time of 8.1 years (IQR = 6.5–10.9). Male sex was associated with higher IUPM (β: +1.2 IUPM), as was being post-menopausal when compared to pre-menopausal women (β: +0.4 IUPM). There was no evidence of viral failure in the cohort. Additionally, there was no evidence of evolution in the outgrowth viruses associated with the DTG-associated increase.

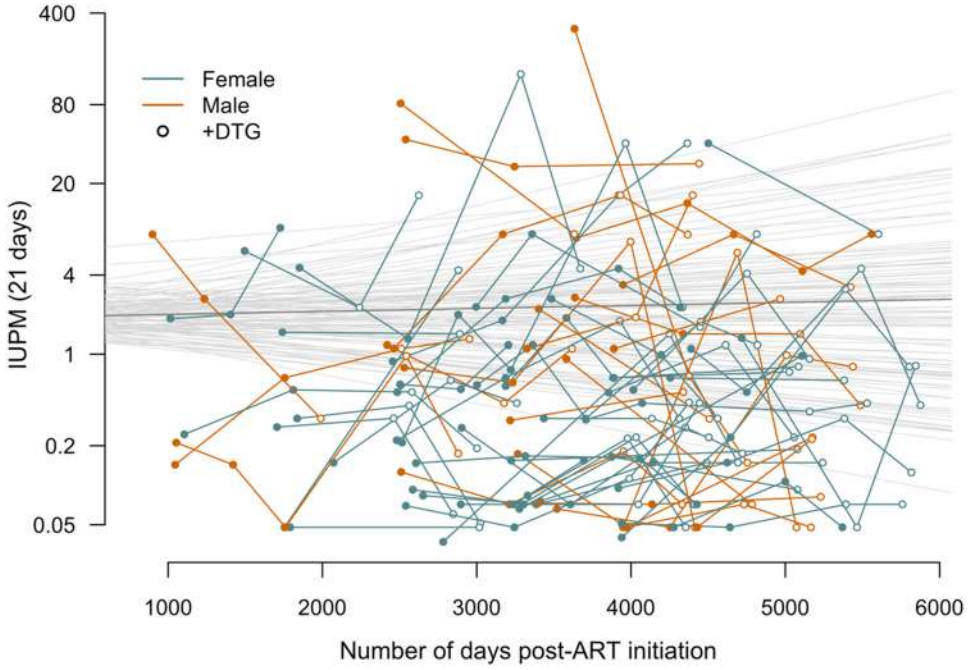

Figure 1 (abstract: PP 7.7–00055) Longitudinal Ugandan IUPM data.

Conclusions: These data suggest that either the initiation of DTG, or cessation of NNRTI, is associated with a significant temporary increase in the circulating inducible replication-competent LVR. This phenomenon, if confirmed in other populations, may provide an opportunity to target the LVR during DTG initiation.

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PP 7.8 – 00060

Slowing or Reversal of Decay of Intact Proviruses Over Two Decades of Suppressive ART

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Background: The intact proviral DNA (IPD) assay is a measure of the replication competent HIV reservoir. Little is known about the decay patterns of IPD in people with HIV (PWH) during long-term (15–20 yr) suppressive ART.

Methods: Participants in ACTG A5321 with chronic HIV and documented suppression of viremia (<50 copies/mL) for >15 yr had measurements of intact, 5′ or 3′ defective, and total proviral DNA from blood samples. A biexponential model for IPD was estimated using non-linear regression.

Results: Fourteen participants (5 female) were evaluated longitudinally from ART yr 1 to ART yr 17–23 (median 20 yr; 8–10 timepoints). Median pre-ART plasma HIV RNA was 4.2 log10 cp/mL and median pre-ART CD4 count was 377/mm^3. At yr 1 of ART, median IPD was 204 cp/million CD4+ T cells; median (Q1, Q3) IPD percentage (intact/total) was 66% (41, 83). By a median of 20 yr on ART, IPD percentage had decayed to 7% (4, 10) reflecting selective decay of intact proviruses. Five participants had biphasic IPD decay, 3 had biphasic decay with a second phase plateau (slope effectively zero), and 2 showed evidence of increased IPD levels during the second decade (Fig. 1). The inflection or transition of decay occurred at a median of 5 yr after ART initiation (range 2–13 yr). The median first phase IPD half-life was 1 yr (n = 10), whereas the median second phase IPD half-life was >25 yr (n = 8). For the two participants with late IPD increases, second phase half-life was undefined. In the other 4 participants, there was a variable pattern of IPD decay, perhaps due to fewer cells assayed or lower IPD levels.

Conclusions: In PWH on long-term suppressive ART, three patterns of IPD decay were revealed: (1) biphasic decline with markedly slower second phase decline; (2) initial decline that transitions to a zero-slope plateau; and (3) initial decline followed by late increases in IPD. The mechanisms of slower second phase decay or reversal are uncertain but may include the inability to clear cells with intact but transcriptionally silent proviruses and clonal expansion of cells with intact proviruses.

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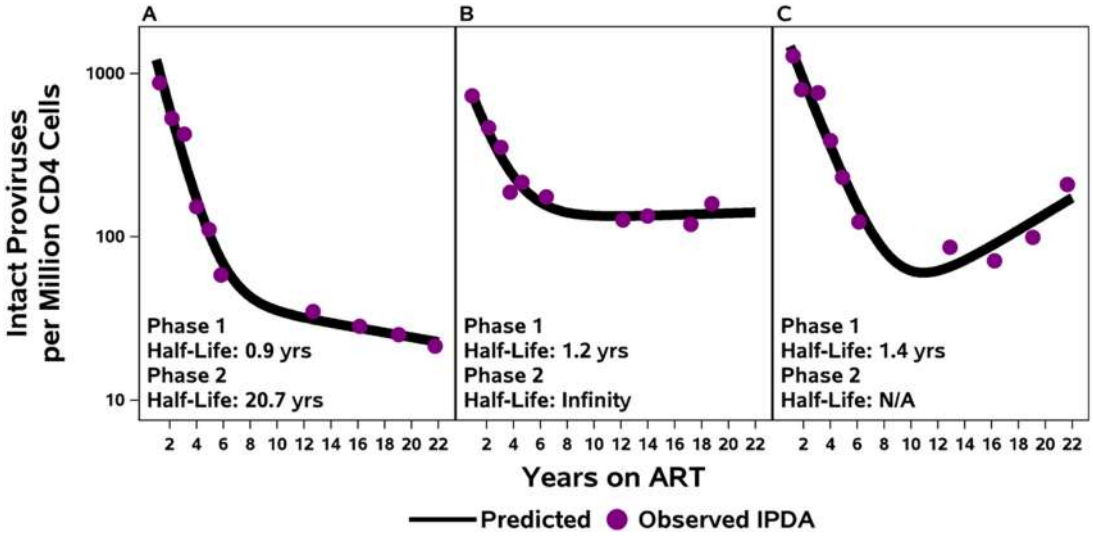

Figure 1 (abstract: PP 7.8–00060) Examples of decay patterns in intact proviral DNA.

PP 7.9 – 00073

HIV-1 Cell-Associated RNA Provides 0.8 Prediction Accuracy for Time to Rebound after Treatment Interruption

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Background: The goal of ACTG A5345 cohort IMAP study was to identify pre-treatment interruption (pre-TI) biomarkers predictive of time to HIV viral rebound to support the design and evaluation of HIV cure trials.

Methods: Total RNA was isolated from A5345 participant pre-TI PBMCs (N = 45: N = 33 initiated ART in chronic HIV-1 (chronic cohort); N = 12 initiated ART in acute/early HIV-1 (acute/early cohort)). HIV RNA targets were amplified and detected using oligonucleotide-templated photoreduction with fluorogenic probes (Jan Biotech, Inc.) specific to conserved HIV-1 sequences. Spearman correlations and cross-validation-based predictions were performed.

Results: TAR, long-LTR, Pol and Env cell-associated HIV-1 RNAs were tested, and we found TAR RNA level strongly correlates with time to rebound for the A5345 acute/early cohort (−0.67, p = 0.02), suggesting TAR RNA is largely produced by the functional reservoir in this cohort. While ART initiation fixes the HIV provirus population, ART duration progressively selects against functional proviruses and loss-of-function mutations accumulate. We found controlling for ART duration improves the TAR RNA level to rebound correlation and significance (−0.75, p = 0.007).

Most significantly, we found that for the early/acute cohort, ART duration together with TAR RNA level provides 0.79 prediction accuracy (p = 0.018) of time to rebound. No other measure used in the trial approaches this predictive power (for the acute/early cohort, iSCA correlated at −0.68, p = 0.015, but with only 0.16 prediction accuracy). Correlation provides a comparison of known variables; predictive power indicates future time to rebound.

Conclusions: We report that HIV-1 cell-associated RNA provides 0.8 predictive accuracy for time to rebound in the ACTG A5345 acute/early cohort. The data indicate that the size of the functional reservoir in this cohort is adequately captured by the cell-associated TAR RNA level. The high predictive value and reproducibility of the assay for the acute/early cohort may be useful for clinical trials evaluating new HIV remission and cure treatments. The strong predictive accuracy of the assay supports small (N£20) Phase II clinical drug evaluation groups composed of acute/early participants.

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PP 7.10 – 00088

Macrophage-Tropic HIV-1 Variants Contribute to Pediatric Rebound Viremia off ART

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Background: HIV-1 latent reservoir studies have focused on peripheral blood CD4+ T cells. HIV-1 also infects macrophages and infected macrophages persist on suppressive ART. While tissue-based reservoirs are difficult to study, analyzing plasma rebound viruses can provide insight into these reservoirs and their susceptibility to pharmacologic or immunologic control. Macrophage-tropic (mac-tropic) viruses have been detected in infant plasma as early as 2 months of age (Peters, J Virol, 2006), suggesting the early establishment of macrophage reservoirs in children. The present study analyzed rebound plasma virions from a very early-treated child who subsequently discontinued ART to evaluate the extent to which macrophages serve as a reservoir and contribute to rebound viremia.

Methods: We have previously reported a well-documented case of HIV-1 remission in a child who initiated ART at 30 hours (Persaud, NEJM, 2013); plasma taken at the viral rebound off ART (46 months of age; Luzuriaga, NEJM, 2015) was used for analyses. Single genome amplification was used to generate full-

length env sequences directly from rebound plasma virions, or after virion immunocapture (VIC) using CD3- or CD14-conjugated magnetic beads. Env+ pseudoviruses (Env+PV) were then constructed and used to measure infection of TZM-bl cells or primary macrophages, as well as susceptibility to inhibition by neutralizing Ab and soluble CD4 (sCD4).

Results: A subset of Env+PV generated directly from rebound virions infected primary macrophages (mac-tropic) and were sensitive to sCD4 inhibition. Env+PV prepared from CD14-captured virions were mac-tropic and had higher sensitivity (lower IC50's) to sCD4 inhibition, while Env+PV from CD3-captured virions were non-mac-tropic and had lower sensitivity (higher IC50's) to sCD4 inhibition. The study of additional very early-treated children is ongoing.

Conclusions: Mac-tropic viruses contributed to HIV-1 rebound. The VIC results suggest that mac-tropic viruses likely originated from macrophage-lineage cells and are compatible with prior reports of early macrophage reservoir establishment in pediatric infection.

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PP 7.11 – 00091

In vivo and in vitro imaging of viral reservoirs to understand bystander damage during chronic HIV infection

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Background: HIV has become a chronic disease due to the success of antiretroviral therapy (ART), but ART is not a cure. The main obstacle to achieving HIV eradication is the early generation of circulating and tissue-associated viral reservoirs. The best characterized viral reservoirs are memory CD4+ T lymphocytes due to easy access to blood. However, it has become evident that tissue-resident viral reservoirs, including in lymphoid and brain tissues, are essential to reach a cure.

Methods: Recently, our laboratory developed a multi-detection system to identify and quantify in the same assay HIV-DNA, viral mRNA and several host/viral proteins using in situ hybridization in combination with antibody staining.

Results: Our microscopy and image analyses identified in the brain and lymph nodes of HIV-infected individuals under long-term ART three different cell populations or compromised areas: first, the viral reservoir with HIV integrated DNA; second, uninfected cells surrounding viral reservoirs up to a distance of ~350–500 μm with significant signaling, immune, and inter-organelle compromise and lastly, cells surrounding viral reservoirs to distances more than 500 μm that looks healthier. These three distinct areas will be examined in the current proposal.

Our data using in situ hybridization and antibody staining of human brain tissues indicates that in lymph nodes, the viral reservoir corresponds to T cells and a small subset of dendritic cells. Both reservoirs were sensitive to long-term ART (up to 23 years of treatment); however, long-term drug abuse prevented the reduction in the viral reservoir pool achieved by ART. The blood samples identified a similar profile for T cells and monocytes. In the brain, we identified that microglia/macrophages and a small percentage of astrocytes are the reservoirs. Long-term ART reduced the myeloid reservoirs but did not affect the astrocyte reservoir, suggesting that some viral reservoirs are insensitive to long-term ART. Drug abuse prevented the reduction in the CNS reservoir pool induced by ART in myeloid but not glial cells.

Conclusions: Our data indicate that HIV and drug abuse potentiate to prevent long-term viral reservoir clearing.

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PP 7.12 – 00192

The immune synapses reveal aberrant functions of CD8 T cells during chronic HIV infection

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Background: Cytotoxic CD8 T cells play essential role in anti-HIV immunity. Chronic HIV infection causes persistent low-grade inflammation that induces premature aging of the immune system in the infected people including senescence of memory and effector CD8 T cells. Upon recognition of the infected cells, CD8 T cells establish contact area which transforms into highly organized structure called immunological synapse (IS). The formation of this structure contributes to the coordinated delivery and release of cytolytic granules to target cells hat determined efficiency of the CTL cytolytic activity.

Methods: We exposed freshly isolated T cells to planar lipid bilayers containing ligands for T-cell receptor and a T-cell integrins and analyze the cellular morphology, dynamics of synaptic interface formation and patterns of the cellular degranulation.

Results: The cells with different interfaces exhibited notable dissimilarity in pattern and kinetics of degranulation that was dependent on T cell differentiation stage and the infection status. Unexpectedly, we have found that a substantial fraction of naïve CD8 T cells from HIV-infected people are able to form mature synapses and release granules, a signature of a differentiated T cells.

Conclusions: Our findings suggest that chronic inflammation during HIV infection mediates changes in the ability of T-cells to form synaptic interfaces and, consequently, alters their functional activity. Further differentiation of aberrant naïve T cells may lead to the development of anomalous effector T cells undermining their capacity to control HIV and other pathogens that could be contained otherwise.

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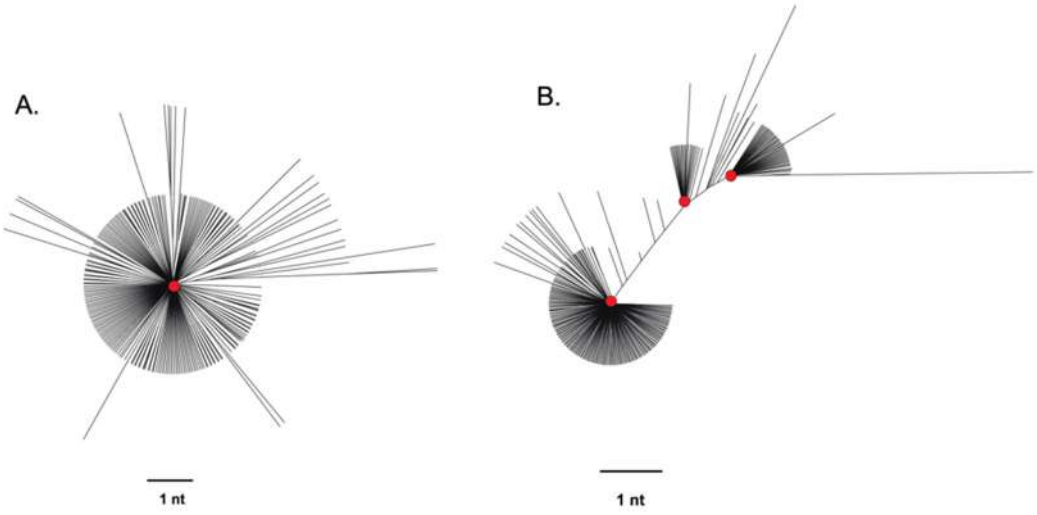

Figure 1. Star phylogeny in donor in Fiebig III after transmission of a single founder (A). Phylogeny in donor in Fiebig II after transmission of multiple founders (B). The red circles show the consensus sequences of the transmitted founders.

Figure 1 (abstract: PP 7.13–00090) Phylogenetic Analyses.

PP 7.13 – 00090

Early evolution of HIV-1 from transmitted founders during acute infection

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Background: To better define the number of transmitted founder variants and sequence divergence from founders in the first few weeks following HIV-1 transmission, we performed ultrasensitive single genome sequencing (SGS) of pol and env HIV-1 RNA in plasma samples from individuals with acute HIV-1 infection.

Methods: HIV-1 RNA was extracted from plasma of donors with acute CRF01_AE infection (Fiebig II-IV) enrolled in the RV254/SEARCH070 Cohort (NCT00796146). Five donors had been identified by standard SGS as having multiple transmitted founders (TFs) and 10 as single TFs, consistent with the prior report showing 70% having single TFs in RV254. Our ultrasensitive SGS method with primer IDs and paired-end Illumina sequencing was applied to identify >10 000 independent pol and env sequences per sample. We used radial trees (Figure 1) and star phylogeny to identify TFs, calculated transition/transversion (Ti/Tv), non-synonymous/synonymous (dN/dS) ratios, identified drug resistance mutations (DRMs), and analyzed the phylogenetic relationships between emergent variants.

Results: An average of 12 823 pol and 11 249 env independent RNA sequences determined from consensus building of millions of barcoded reads were obtained from the plasma of the 15 donors. In single TF infections, an average of 85% of the genomes were identical to the TF. An average of 13% of the genomes contained only a single mutation, 1% contained 2 mutations and 1% had 3–6 mutations. Ti/Tv ratios were 5.5 and 3.4 in pol and env respectively. DRMs were found at very low frequency (0.01–0.09%) in all samples. Mutations predicted to confer resistance to the antibody VRC-01 were also present in all samples. The dN/dS in env ($M = 2.83$, $SD = 0.90$) was greater than pol ($M = 1.47$, $SD = 0.74$; $t(9) = 3.31$, $p = 0.009$). Mutation frequency was higher in both genomic regions in samples from later Fiebig stages.

Conclusion: Ultrasensitive SGS (>10 000 genomes per sample) did not identify more transmitter founder variants than low depth (10 genomes) SGS, verifying that HIV-1 infection is most commonly established by a single variant. By contrast, ultrasensitive SGS revealed viral evolution from the founder variants in all samples including those from as early as Fiebig stage II, indicating rapid evolution of HIV-1, including appearance of DRMs soon after transmission.

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Session 8: Antibody & Immune based Therapies

OP 8.2 – 00033

Interleukin-2 administration is a potent latency reversal agent in people with treated HIV infection

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Background: Interleukin (IL)-2 activates and expands T cells and NK cells, promotes NK cytotoxicity, and *in vivo* in uncontrolled HIV infection, increased plasma HIV levels. We asked whether administration of recombinant (r)IL-2 to people with HIV (PWH) well-controlled on antiretroviral therapy (ART) would induce HIV replication and activate cellular immunity, potentially leading to reduction in the HIV reservoir.

Methods: Nine men with ART-suppressed HIV were enrolled to examine the effects of eight 4-day cycles of 5 million units twice-daily subcutaneous rIL-2 administration on levels of replication-competent HIV in circulating CD4 T cells. All nine participants completed the first 4-day cycle, and some received further cycles of rIL-2. Plasma cytokine levels were quantified, and cells were analyzed by flow cytometry and scRNA-seq/Ab-seq for markers of T and NK cell activation at day 7 of the first cycle, and at later timepoints. Plasma viral levels were measured by clinical and ultrasensitive viral load assays. Levels of HIV in CD4 T cells were measured by intact proviral DNA assay.

Results: After rIL-2 administration CD4 and CD8 T cell counts rose; "Treg-like" CD25 + CD127− CD4 T cell proportions increased; cycling of T cells increased; transcriptomic signatures of enhanced cytolytic activity (e.g. *IL32*, *GZMK*, *GNLY*) increased; proportions of NK cells expressing NKG2A and CCR2 increased; and numerous inflammatory mediators, acute phase proteins, and coagulation markers increased. Plasma HIV levels rose from <20 copies/mL by clinical assay to a median of 57 copies/mL (range 20–1635 copies/mL; $P = 0.047$), and similar results were obtained with ultrasensitive assays. The study was terminated in consultation with the safety monitoring committee because of toxicities in three participants, one with capillary leak syndrome requiring hospitalization and two with biochemical hypothyroidism.

Conclusions: IL-2 administration was not well-tolerated in this small study among PWH with controlled infection but did promote T and NK cell activation and induced substantial latency reversal. After one cycle, there were not clear effects on HIV reservoir size in circulating CD4 T cells. Further study of IL-2 strategies in HIV cure research is warranted.

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OP 8.3 – 00028

HIV-vaccine induced, broad and polyfunctional CD4 and CD8 T cell responses are associated with prolonged time off ART and lower pVL at the end of ATI in the AELIX-002 therapeutic vaccine trial

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Background: The AELIX-002 trial was a randomized, placebo-controlled trial to evaluate the safety, immunogenicity and effect on viral rebound during an analytical treatment interruption (ATI) of a combination of DNA.HTI (D), MVA.HTI (M) and ChAdOx1.HTI (C) vaccines in 45 early-treated individuals (NCT03204617). Multiparametric flowcytometry was used to characterize HTI-vaccine induced responses in detail and to relate their profile to ATI outcomes.

Methods: Cryopreserved PBMCs from week 28 (4 weeks after completion of DDDMM-CCM and 4 weeks before starting the ATI) were stimulated with 4 peptide pools (9–43 peptides each) spanning p17, p24/p15, Pol and Vif/Nef regions included in the HTI vaccine immunogen. Surface markers of T cell linage (CD3/CD4/CD8), follicular T cells (CXCR5/PD1), phenotype (CD45RA/CCR7), activation (CD69/HLADR) and exhaustion (TIGIT/PD1) were measured, along with effector functions assessed as production of INF-γ, GrazymeB, IL-2 and TNF-α.

Results: HTI-specific responses, defined as the sum of the HTI-IFN-γ+ populations for each HTI peptide pool, were both CD4 and CD8 T cell-mediated. Vaccinees had a higher frequency of HTI-specific polyfunctional CD8 T cells (expressing IFN-γ/GzmB or IFN-γ/GzmB/TNF) and CD4 T cells (predominantly expressing combinations of IL-2, IFN-γ and TNF-α) than placebos. T cell exhaustion markers were not increased in HTI-specific T cells in vaccinees after completing DDDMM-CCM. Importantly, the frequency of CD8+ -and to a lesser extent CD4+- T cells, expressing GzmB+ was positively correlated with the time off ART and with lower HIV-1 pVL at the end of ATI in vaccinees without beneficial HLA class I alleles, but not in placebo recipients.

Conclusions: Vaccination induced the desired polyfunctional CD4 and CD8 T cell responses to HTI that correlated with efficacy outcomes during an ATI in vaccinees without beneficial HLA class I alleles.

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OP 8.4 – 00165

TLR agonist and SIV mAbs administered to SIV-infected ART-suppressed macaques did not delay rebound after treatment interruption

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Background: Elimination of HIV-1-infected cell reservoirs through HIV monoclonal antibody (mAb) therapy is actively being explored in HIV-1 curative strategies. Combination approaches involving toll-like receptor (TLR) agonists and mAbs have shown success in limiting viral rebound following analytical treatment interruption (ATI) in SHIV-infected macaques. Here, we sought to test the therapeutic efficacy of neutralizing rhesus SIV mAbs in combination with TLR agonists in SIV-infected macaques, a robust pre-clinical model with consistent viral replication that suppresses with ART, enabling evaluation of HIV-1 cure strategies in a potentially more stringent manner than SHIV-based models.

Methods: 48 rhesus macaques (6 groups of n = 8 animals) were inoculated intravenously with the tier 2 swarm SIVmac251 and ART was initiated 7 days post-infection. Four groups received repeated administrations of one of four TLR agonists: (1) 2BXy (TLR7/8); (2) CpG (TLR9); (3) LPS (TLR4); or (4) intravenous BCG (TLR2/4/9), at weeks 32–50 while viremia was suppressed. In addition, animals received 6 administrations of 2 anti-SIV mAbs (weeks 40–50) including a potent CD4bs mAb. Additional groups included: (5) mAbs without TLR agonists; and (6) ART only. ATI occurred at week 76.

Results: TLR agonist administration stimulated strong immune activation, with elevated CD69 expression on T and NK cells for all tested TLR agonists, and a large increase in Ki67 following BCG vaccination (~50% Ki67+ T and NK cells one week post-inoculation). Despite substantial T cell activation, viral blips were not observed during TLR agonist administration. 37.5% of animals that received TLR agonists developed anti-drug antibody responses against the infused rhesus mAbs; responses were not observed in the mAb-only arm. Following ATI, viremia rebounded in all animals within 4 weeks, with no difference in time to rebound or setpoint viremia among groups.

Conclusions: Co-administration of anti-SIV mAbs and TLR agonists (including a TLR7 agonist) during ART- suppressed SIV infection did not impact time to viral rebound or setpoint viremia following ATI, in contrast to previous studies evaluating HIV mAbs in a SHIV model. The mechanisms underlying these discordant outcomes are unclear, supporting further investigation of immunologic and virologic endpoints achieved by mAb plus TLR agonist therapy associated with post-ATI control.

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OP 8.5 – 00104

TGF-beta Blockade to Stop HIV White Noise: a New "Release and Kill" HIV Strategy

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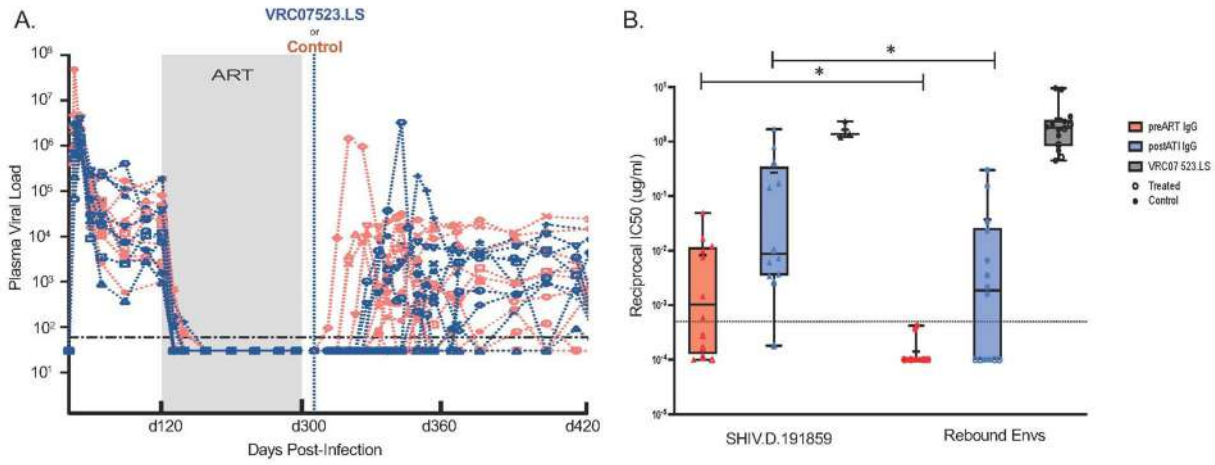
Background: We recently demonstrated that TGF-β inhibits HIV-1 reactivation in vitro and blocking TGF-β signaling with a small molecule, galunisertib ((LY2157299; Gal) that reached clinical phase 2 with Eli Lilly, reactivated HIV/SIV from latency ex vivo



A. Study Schema. 18 RM were IV inoculated with SHIV.D.191859 and started on ART at day 120. After 6 months ART, 9 RM were given 30 mg/kg VRC07.523LS and all RM underwent ATI. VRC07.523LS-treated RM experienced delayed rebound (median 41 vs. 28 days, p=0.0164). **B. Autologous neutralizing antibody responses vs. inoculum SHIV.D.191859 and each RM's rebound Env**. Reciprocal IC50 on Y-axis and responses against SHIV.D shown as triangles on left and against Rebound Envs as circles on right. Pre-ART (day 120) IgG responses shown in red, 8-weeks post-ATI (day 360) shown in blue, VRC07.523LS responses in black.

Figure 1 (abstract: OP 8.6–00166)

and in vivo. Moreover, a short 1–2 week Gal treatment in ART-suppressed SIV-infected rhesus macaques stimulated anti-SIV T cell responses.

Methods: To confirm and expand our findings, 7 rhesus macaques were infected intravenously with the barcoded SIVmac239M2 and treated with ART from week 6 post-infection (pi). After 29 weeks on ART, the macaques were treated with a more extensive Gal regiment consisting of four 2-weeks cycles of oral Gal (20 mg/kg) twice per day. Blood, lymph nodes and mucosal samples were collected before and after each of the first 3 treatment cycles and at the end of the 4th cycle. PET/CT imaging using the ^{64}Cu-DOTA-F(ab)$_2$ p7D3 probe were performed before and after the first 3 Gal cycles. ART was interrupted 3 weeks after the end of the 4th cycle and the macaques euthanized 7 weeks later.

Results: Gal treatment induced viral reactivation in blood with blips above 100 copies/ml in 5 out of the 7 macaques. Extensive SIV reactivation in tissues was documented by PET/CT in all macaques particularly after cycle 2. Immunophenotyping and barcode analysis is undergoing. However, preliminary data suggest that Gal stimulated an effector phenotype in T cells with increased expression of CD95 and shedding of CD62L. However, Gal did non induce systemic immune activation at least at the end of the 1st cycle, as suggested by PBMC RNAseq data and lack of increased expression of CD69 and CD38 on T cells. Finally, cell-associated viral DNA (vDNA) decreased by over a Log in lymph nodes and colorectal biopsies of all treated macaques by the end of Gal cycle 4th compared to before Gal.

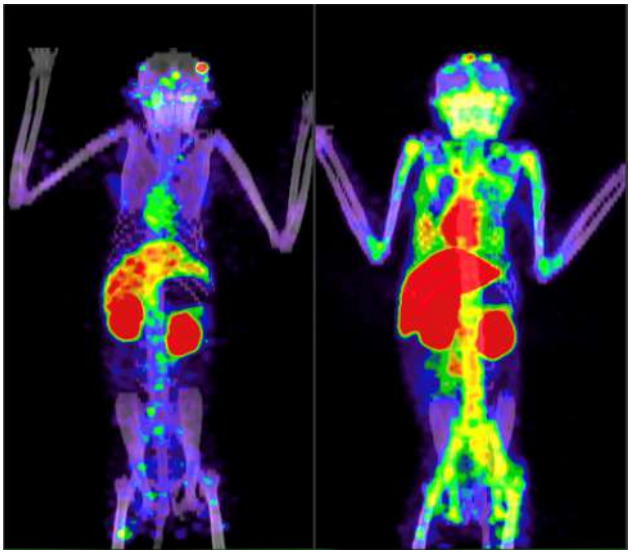


Figure. Before and after cycle 2 of galunisertib.

Conclusions: Our studies suggest that targeting TGF-β in vivo leads to attrition of the viral reservoir during ART, particularly in tissues, by a combination of an increased frequency of SIV reactivation events and enhanced SIV-specific responses.

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OP 8.6 – 00166

Autologous neutralizing antibody responses in bnAb-treated rhesus macaques

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Background: Broadly neutralizing antibodies (bnAbs) are important tools for HIV-1 cure strategies, yet the role of autologous neutralizing antibodies (anAbs) during bnAb therapy is unclear. We studied the kinetics of anAb responses during VRC07523.LS therapy at analytical treatment interruption (ATI) in a robust SHIV-infected Rhesus Macaque (RM) model of HIV-1.

Methods: 18 RMs infected IV with 1×10^6 infectious units of SHIV.D.191859 (SHIV.D) initiated ART at 120 days post-infection (dpi). After 6 months of ART, all 18 RM underwent ATI, during which 9 were administered 30 mg/kg VRC07.523LS. Plasma virus env sequences were derived by single genome sequencing. SHIV.D and rebound Envs were tested for neutralization sensitivity.

Results: 18 RM experienced median peak and pre-ART viral loads of 4.91×10^6 and 3.94×10^4 copies/mL, respectively. Virus was suppressed within 3 weeks of ART and rebounded in all RM following ATI, with a significant delay in VRC07.523LS-treated RM (median 41 vs. 28 days, $p = 0.016$). At 120 dpi, 56% (10/18) of RMs had anAb responses against SHIV.D, with median 50% inhibition of 1:47 plasma dilutions (ID50) and 87 μg/mL IgG (IC50), with strong correlation between plasma and IgG ($r_s = 0.85$, $p = 1E\text{-}5$). AnAb responses were stable over 6 months of ART. Plasma virus env sequenced pre-ART and at rebound (median 31 sequences per RM) revealed 1–5 rebound virus lineages, which were universally resistant to 120 dpi plasma IgG. Eight weeks post-rebound, plasma anAb responses rose ~1000-fold against both SHIV.D and rebound Envs, with greater magnitude vs. SHIV.D ($p = 0.0053$). In treated RMs, plasma VRC07523.LS was detectable at rebound in 7/9 RM and undetectable 8 weeks post-ATI in 9/9. Rebound Envs in bnAb-treated RM were more VRC07523.LS-resistant than controls ($p = 0.0316$).

Conclusions: SHIV.D-infected RMs demonstrate viral kinetics, anAb responses, and response to bnAb monotherapy that mirrors humans. Rebound virus escaped baseline anAb responses, suggesting ongoing humoral immune pressure at ATI. Virus rebound preferentially boosted anAbs against inoculum vs. rebound virus, indicating antibody imprinting. AnAb potency and durability suggest a potential role in restricting archived virus to complement bnAb-based interventions.

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PP 8.1 – 00003

Optimization of the 5′ cap and untranslated regions enhances the immunogenicity of an mRNA-based therapeutic vaccine in SIV-infected rhesus macaques on ART

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Background: Although antiretroviral therapy (ART) has dramatically improved the life expectancy of people living with HIV/AIDS (PLWHA), ART alone cannot cure HIV infection. We evaluated whether a messenger ribonucleic acid (mRNA)-based therapeutic vaccine can be used to enhance CD8+ T cell immunity towards the goal of reducing HIV reservoirs during ART and control of viral replication after ART interruption.

Methods: Utilizing the nucleoside-unmodified RNActive® vaccine platform, we found that an mRNA construct expressing SIV Gag formulated in lipid nanoparticles (mRNA/SIV-Gag LNP) is highly immunogenic in SIV naïve rhesus macaques (RM), eliciting potent, broad, and systemically distributed SIV-specific CD8+ T cell responses. Here, we assessed whether modification of the 5′ cap as well as 5′ and 3′ untranslated regions (UTRs) can further enhance the immunogenicity of mRNA/SIV-Gag LNP in SIV-infected RM on ART. A total of 9 RM were IV inoculated with SIVmac239 and started on ART 9 days post-infection (dpi). RM were subsequently randomized into 3 groups (n = 3) that received 100 ug IM injections of (a) the standard mRNA/SIV-Gag LNP, (b) an optimized (CAP/UTRs) mRNA/SIV-Gag LNP, or (c) PBS as control at weeks 30, 33, 36, 60, and 93 post-ART initiation. RM were euthanized on ART, 2 weeks after the last vaccination to assess the distribution of vaccine elicited CD8+ T cell responses.

Results: Two-fold higher frequencies of Gag-specific CD8+ T cells were observed in bronchial alveolar lavage of RM that received the optimized mRNA/SIV-Gag LNP relative to those that received the standard mRNA/SIV-Gag LNP. Higher Gag-specific CD8+ T cell responses in blood and lymph nodes were also observed in the optimized mRNA/SIV-Gag LNP vaccinees. Responses were higher in both vaccine groups compared with PBS controls. Finally, the magnitude of CD8+ T cell recognition and suppression of viral replication in ex vivo studies using autologous SIV-infected CD4+ T cells was higher in RM that received the optimized mRNA/SIV-Gag LNP construct.

Conclusions: Collectively, these data indicated that optimization of the 5′cap and untranslated regions of the mRNA/SIV-Gag LNP can enhance the magnitude and functional activity of SIV-specific CD8+ T cells in SIV-infected RM on ART.

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PP 8.2 – 00015

Retargeting cytomegalovirus-specific CD8+ cytotoxic T lymphocytes to kill HIV/SIV-infected cells via peptide-MHC I-antibody fusion proteins

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Background: A sterilizing cure for HIV will likely require cytolytic effector cell-mediated elimination of HIV-infected cells. CD8+ cytotoxic T lymphocytes (CTLs) are highly efficacious during acute infection but become impaired by escape and exhaustion during chronic infection. By contrast, cytomegalovirus (CMV)-specific CTLs remain functional and refractory to exhaustion, even amidst the generalized immune dysfunction that can occur in chronic HIV infection. Furthermore, CTLs specific for immunodominant CMV-derived peptides are ubiquitous in CMV-seropositive individuals and often reach high frequencies. In this study, we employ the SIVmac239 model of HIV infection in Indian rhesus macaques (RMs) to evaluate the efficacy of peptide-MHCI-antibody (pMHCI-mAb) fusion proteins in facilitating the killing of SIVmac239-infected CD4+ T cells by rhesus CMV (RhCMV)-specific CTLs.

Methods: We generated pMHCI-mAb fusion proteins containing an SIVmac239 envelope-binding molecule (K11 mAb or eCD4-Ig) and Mamu-A*02 loaded with RhCMV IE-1 VY9. Fusion protein efficacy was assessed by flow cytometry-based assays involving co-culture of RhCMV IE-1 VY9-specific CTLs and autologous SIVmac239-infected CD4+ T cells to assess infected cell elimination (live/dead stain, intracellular SIV Gag p27) and CTL activation (CD107a and IFN-γ).

Results: Frequencies of SIV+ target cells were two- to five-fold lower in fusion protein-treated co-cultures than in co-cultures treated with eCD4-Ig alone or the K11 mAb alone. Both the eCD4-Ig fusion protein and K11 fusion protein exhibited potent activity, with EC50 values of 0.99 nM and 4.88 nM, respectively, at an effector-to-target (E:T) ratio of 3:1. Fusion protein-mediated reductions in SIV+ target cell frequencies were statistically significant at both 10 nM and 100 nM ($P < 0.05$ by Welch's t-test). Both fusion proteins facilitated similar reductions in SIV+ target cell frequencies across E:T ratios of 1:1, 3:1, and 6:1, at a constant concentration of 100 nM. Both fusion proteins stimulated CTL degranulation (CD107a) and IFN-γ production

(approximate four-fold increase in the frequency of responding CTLs), while the parental eCD4-Ig and K11 mAb did not.

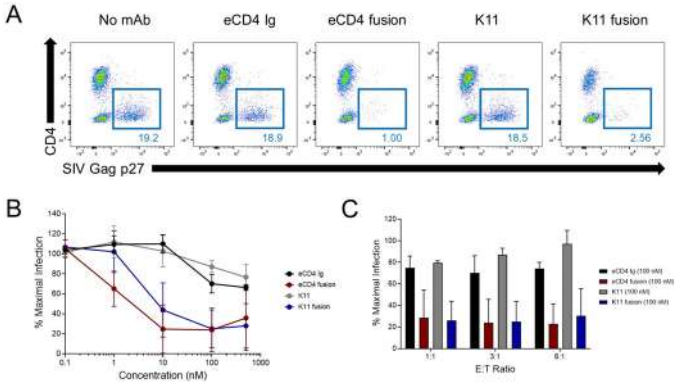

Figure. Fusion proteins facilitate killing of SIV+ cells.

Conclusions: Our findings demonstrate in vitro proof-of-concept for pMHCI-mAb fusion protein-mediated retargeting of CMV-specific CTLs to kill SIV-infected cells and provide a potential strategy to eliminate HIV/SIV-infected cells in vivo.

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PP 8.3 – 00049

Reduction in Markers of HIV Persistence with Gag/Pol/Il-12 DNA Therapeutic Vaccination

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Background: T cell-based therapeutic vaccination is a potential approach to achieving durable control of HIV. We previously reported immunogenicity data from a randomized clinical trial ("PENNVAX," NCT03606213) of Gag + Pol + IL-12 DNA (G/P), Gag + Pol + Env + IL-12 DNA (G/P/E), or placebo (PBO) vaccination in 45 participants with HIV on suppressive antiretroviral therapy (ART), and found that vaccination induced de novo and boosted Gag-specific T-cell responses, with the most robust responses with G/P. We now present virologic outcomes from the trial.

Methods: Participants (median 8.4 years virologically suppressed) were vaccinated Weeks 0, 4, 8, and 12. Markers of HIV persistence were assessed serially pre- and post-vaccination by LTR-gag HIV-1 DNA (total DNA), cell-associated LTR-gag RNA (caRNA) and intact proviral DNA assay (IPDA) on isolated CD4+ T cells from cryopreserved PBMCs, as well as plasma HIV-1 RNA (vRNA) by automated single-copy assay (SCA). Longitudinal changes from baseline to Week 64 were compared between arms by linear mixed effects models and a parametric model accommodating left censoring at limit of detection for change in SCA. Two participants with known ART interruptions were excluded from analyses.

Results: G/P recipients had numerically greater reductions in total HIV-1 DNA, defective genomes, caRNA, and plasma vRNA, and increases in the proportion who achieved an undetectable plasma vRNA over time, but no change in intact proviruses by IPDA (Table). The effects were modest and significant compared to placebo only for total DNA and quantitative plasma vRNA.

	Change from baseline to Week 64			Between-arm p-value*		
Assay	G/P total n=12	G/P/E total n=16	PBO total n=15	G/P vs PBO	G/P/E vs PBO	G/P vs G/P/E
Total HIV DNA, mean (SE) log10 change copies/10^6 CD4	-0.20 (0.10)	-0.07 (0.09)	0.08 (0.09)	**0.04**	0.25	0.33
Intact provirus, mean (SE) log10 change count/10^6 CD4	0.00 (0.10)	-0.11 (0.08)	0.02 (0.08)	0.88	0.23	0.35
Total (3'+5') defective provirus, mean (SE) log10 change count/10^6 CD4	-0.12 (0.06)	0.05 (0.05)	0.02 (0.06)	0.11	0.62	**0.03**
3' defective provirus, mean (SE) log10 change count/10^6 CD4	-0.12 (0.07)	-0.01 (0.06)	-0.02 (0.06)	0.09	0.26	0.22
5' defective provirus, mean (SE) log10 change count/10^6 CD4	-0.04 (0.10	0.17 (0.09)	0.05 (0.10)	0.52	0.34	0.11
caRNA, mean (SE) log10 change copies/10^6 CD4	-0.26 (0.13)	0.02 (0.11)	-0.10 (0.12)	0.34	0.46	0.09
caRNA/total DNA, mean (SE) change	-0.39 (1.11)	0.52 (0.99)	0.85 (0.99)	0.41	0.81	0.54
SCA, mean change (SE) log10 copies/mL	-0.30 (0.10)	0.08 (0.13)	0.18 (0.15)	**0.01**	0.62	**0.02**
SCA, change in proportion of participants with undetectable plasma vRNA	+21% (29% baseline vs 50% W64)	-3% (28% baseline vs 25% W64)	+7% (33% baseline vs 40% W64)	N/A	N/A	N/A

Table. Change in HIV persistence markers by arm

Conclusions: We found modest but consistent signals of reductions in markers of HIV persistence with G/P vaccination, the arm that induced the greatest Gag-specific T-cell responses by IFNg ELISPOT. Further work to characterize correlates of favorable vaccine-elicited immunologic and virologic responses is ongoing, and may inform future therapeutic vaccine design.

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PP 8.4 – 00061

Bispecific antibodies promote natural killer cell-mediated elimination of the HIV reservoir

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Background: The persistence of long-lived HIV-infected cells comprising the latent reservoir is the main barrier to a cure. "Shock and kill," a strategy for clearing the latent reservoir, involves selective reactivation of HIV gene expression through treatment with a latency reversing agent (LRA) followed by immune-mediated elimination of HIV-infected cells. To ensure effective elimination of HIV-infected cells in the context of

"shock and kill," novel immunotherapies must be developed to enhance HIV-specific cell-mediated cytolytic activity.

Methods: Here, we report single-chain diabody (scDb) constructs that target the HIV envelope protein (Env) and the human type III Fcγ receptor (CD16a). Two HIV-1-specific scDbs were designed based on PG16 and 3BNC117, broadly neutralizing antibodies specific for the V1/V2 and CD4 binding site regions of Env respectively. We evaluated the ability of the scDbs to promote HIV-specific natural killer (NK) cell activation and NK cell-mediated cytolytic killing of infected cells in vitro. Using the intact proviral DNA assay and viral outgrowth assays, we quantified changes in the frequencies of infected CD4+ T cells isolated from 10 virally suppressed PLWH following ex vivo reversal of latency and co-culture with autologous NK cells in the presence of the scDbs.

Results: Both scDbs promoted robust and HIV-specific NK cell activation and lysis of infected cells in vitro. These effects were not observed with an isotype control scDb targeting an irrelevant cancer epitope. Further, the Env-specific scDbs mediated remarkable ex vivo clearance of cells harboring intact proviruses (mean reduction 44%, range 20–67%, $p < 0.0001$). This clearance of reservoir cells was highly dependent on efficient latency reversal. Notably, we did not detect changes in cells harboring defective proviruses following ex vivo latency reversal and co-culture, suggesting that the scDbs are highly specific for cells expressing sufficient surface Env. Viral outgrowth assays revealed comparable scDb-mediated reductions in cells harboring inducible, replication-competent proviruses (mean reduction 40%, range 20–61%, $p < 0.001$).

Conclusions: Our study provides evidence that the HIV-specific, NK cell-engaging scDbs described here are capable of mediating potent elimination of HIV reservoir cells. In combination with effective LRAs, the scDbs merit further evaluation as potential therapeutics for use in "shock and kill" HIV cure strategies.

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PP 8.5 – 00080

Fc-engineering of anti-HIV-1 antibodies and nanobodies to improve Fc mediated effector functions

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Background: Broadly neutralizing antibodies (bNAbs) targeting the HIV-1 envelope glycoprotein (Env) have shown potential for the implementation in HIV-1 cure strategies. In addition to the neutralizing capacity, BNabs can mediate Fc effector functions such as complement dependent cytotoxicity (CDC), antibody dependent cellular cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP) to facilitate clearance of HIV-1 infected cells. Research has shown that the Fc effector functions of bNabs contribute substantially to their capacity to block viral entry, suppress viremia and confer therapeutic activity. BNabs may be further modulated through Fc engineering to make them more suitable for therapeutic application. These strategies focus on increasing the affinity for Fc gamma receptors (FcγRs) and C1q to enhance Fc effector functions. Common Fc engineering strategies include: introducing amino acid substitutions, altering the antibody subclass or modulating the N297 glycan.

Methods: We produced eight different variants of the N6, PGT121, PGDM1400, A32, J3 and 1F10 anti-HIV-1 antibodies and nanobody-IgG1 constructs. This panel of antibodies targets various epitopes of the HIV-1 Env and will allow us to study how different engineering strategies affect Fc effector functions in antibodies with different specificities. Our engineering strategies focus on enhancing the binding to FcγRIIA, FcγRIIIA and C1q. This is achieved by producing the antibodies afucosylated, as an IgG3, by elongating the hinge or by introducing amino acid substitutions.

Results: The antibody and nanobody-IgG1 constructs were successfully produced in HEK293F cells and purified after which molecular weight and the composition of the constructs was confirmed using SDS-polyacrylamide gel electrophoresis. We then confirmed that the engineered antibodies retained their ability to bind HIV-1 Env. Additionally, we showed that the engineered antibodies exhibited a higher affinity for FcγRIIA and/or FcγRIIIA. Next, we will study how the different engineering strategies affected our antibodies by looking at C1q binding, neutralization potency and using Fc effector function assays.

Conclusions: This study will show whether the engineering strategies result in epitope specific effects and if the strategies will mediate the same effect in nanobody-IgG1 s constructs as they do in conventional antibodies. If our engineering antibodies demonstrate enhanced Fc effector functions, they may prove to be valuable towards HIV-1 curing strategies.

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PP 8.6 – 00089

Ad26/MVA Mosaic vaccine induced antibody responses with limited cross-reactivity to CRF01_AE infections

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Background: Immune therapies are potential strategies to mediate ART-free control in people living with HIV-1. A heterologous Ad26/MVA Mosaic therapeutic vaccine regimen was tested in Thailand on participants who initiated antiretroviral treatment (ART) during acute HIV-1. After analytic treatment interruption (ATI), this placebo-controlled trial showed no significant difference in time to viral rebound; a post-hoc analysis that excluded a controller in the placebo group showed a modest delay in rebound in vaccinees. We investigated the impact of Ad26/MVA Mosaic vaccination on binding antibody (Ab) responses.

Methods: Binding Abs were profiled in 17 vaccine and 9 placebo participants at five time points between HIV-1 diagnosis in acute infection (prior to ART) and peak viremia post-ATI.

Binding Abs were mapped against Envelope (Env) antigens corresponding to subtypes A, B, C, D and G, CRF01_AE and 02_AG, group M and the Mosaic vaccine. Responses were evaluated using Mann-Whitney tests, Spearman correlations and Cox proportional-hazards models.

Results: After at least two years of ART and prior to vaccination, binding Abs were low and preferentially targeted CRF01_AE antigens (25 participants had Env corresponding to CRF01_AE). Between acute infection and the first vaccination, binding Abs increased in participants who started ART during Fiebig I-III but decreased in participants starting ART during Fiebig IV. Ad26/MVA Mosaic vaccination increased binding Abs in vaccinees compared to placebos ($p < 0.0001$) between pre-vaccination and peak immunity with a median fold change of 15.4 for vaccine matched Env, 14.1 for subtype B, but only 2.8 for CRF01_AE antigens. The magnitude of binding antibodies declined between peak immunity and ATI but Abs were still significantly higher in vaccinees ($p < 0.0001$) at ATI. Upon viral rebound, the magnitude and targets of binding Abs did not differ across treatment groups and were not associated with time to viral rebound.

Conclusions: Ad26/MVA mosaic vaccination elicited Ab responses to multiple subtypes but did not specifically boost the infection-specific CRF01_AE responses, potentially explaining the limited impact of these binding Abs on CRF01_AE viral control post-ATI. The limited cross-reactivity with CRF01_AE emphasizes the need for vaccines that elicit responses representing circulating viruses in a population.

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PP 8.7 – 00098

The sequestration and expansion of effector lymphocytes in lymphoid tissue using combination FTY720 and N-803 immunotherapy at ART initiation fails to limit SIV persistence

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Background: Lymphoid cellular reservoirs are anatomically separated from cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells that control or eliminate virus-producing cells; thereby creating an immune-privileged anatomical niche that contributes to long-term viral persistence during suppressive antiretroviral therapy (ART). We hypothesize that combination immune-based approaches can retain cytolytic $CD8^+$ T cells and NK cells in lymph nodes (LNs) and induce cytotoxic function to facilitate viral control; we therefore investigated the activity of combination FTY720 and N-803 therapy at ART initiation during late acute infection; when the viral reservoir is fully established, and SIV-specific cytotoxic responses are at their zenith.

Methods: 10 rhesus macaques (RMs) were intravenously infected with 300 TCID50 $SIVmac_{239}$ and initiated daily ART (dolutegravir, tenofovir disoproxil fumarate, and emtricitabine) at 22 days post-infection. 5 RMs underwent combination immunotherapy with oral FTY720 (500 μg/kg; daily for 58 consecutive days) started at ART initiation and subcutaneously injected N-803 (100 μg/kg; 4 doses with a 2-week spacing) started 3-days following ART initiation. The remaining 5 RMs served as treatment-naïve, ART-only controls. All animals were euthanized 3 months following therapy cessation.

Results: In LN, there was an increase in the ratio of $CD8^+$ to $CD4^+$ T cells with the former showing a pronounced enrichment in the effector memory compartment ($CD95^+CCR7^-$; T_{EM}), which were more proliferative ($Ki\text{-}67^+$), cytotoxic (Granzyme B^+), and with higher antiviral potential (TOX^+). The proportion of SIV-specific $CD8^+$ T cells were not enriched by the intervention. Additionally, therapy increased the frequency and proliferation of LN NK cells, including those associated with antibody-dependent cellular cytotoxicity ($CD56^-CD16^+$). Despite lower levels of blood $CD4^+$ T cells, treated animals had indistinguishable viral load decay following ART initiation relative to controls and exhibited similar content of LN cell-associated SIV-DNA and -RNA during and following the intervention.

Conclusions: While bioactive and successful in expanding effector immune responses in LN, FTY720 and N-803 therapy nonetheless failed to affect the content of cell-associated SIV-DNA with short-term ART. These results suggest strategies relying on the sequestration of effector immune cells in LN are, by itself, unlikely to contribute to a HIV cure.

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PP 8.8 – 00129

Antibody mediated killing of HIV-1 infected cells with glycoengineered broadly neutralizing antibodies

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Background: Despite the success of anti-retroviral therapy in suppressing HIV-1, alternative approaches are required to achieve ART-free remission or complete eradication of HIV-1 in people living with HIV-1 (PLWH). One such strategy, the shock and kill, aims to reactivate the latently HIV-1 infected cells followed by clearance of these reactivated cells through immune effector cells such as CD8+ T cells and Natural killer (NK) cells. Broadly neutralizing antibodies (bNAbs) targeting the HIV-1 envelope glycoprotein (Env) are interesting candidates to complement the development of an efficient kill strategy as they were found to delay viral rebound in ART treatment interruption (ATI) studies. To further enhance their killing capacity through NK-cells, we produced glycoengineered (afucosylated) bNAbs that have an enhanced affinity for Fc gamma receptor IIIa (FcγRIIIa) on NK-cells.

Methods: Monoclonal anti-HIV-1 bNAbs (N6, 2G12, PGDM1400, PGT121 and PGT151) were produced as conventional and as afucosylated antibodies and characterized for the

degree of fucosylation (mass-spectrometry), HIV-1 Env binding (ELISA), NK-cell activation (CD107 expression, FACS) and killing of HIV-1 infected cells. CD56+ NK-cells were isolated from PBMCs from healthy donors (HD) and PLWH and served as effector cells in NK-cell activation and killing assays. Reactivated ACH-2 cells or HIV-1 infected CEM.CCR5.Nkr cells served as target cells and antibody dependent cellular cytotoxicity (ADCC) was quantified by the loss of infected (p24+) cells after co-incubation with NK-cells and antibodies.

Results: Using mass-spectrometry, we confirmed that the fucosylation degree of the afucosylated antibodies was markedly reduced (0–20%) compared to the conventional bNAbs (>95%). Afucosylated antibodies displayed enhanced FcγRIIIa binding, NK-cell degranulation and NK-cell activation compared to conventional bNAbs. Furthermore, ADCC of reactivated latent cells was observed with three afucosylated anti-HIV-1 antibodies (N6, 2G12, PGT151). When NK-cells from PLWH were used, the activation and degranulation response to afucosylated antibodies was less prominent, which was partially explained by low CD16 expression.

Conclusions: Here, we demonstrate that afucosylated anti-HIV-1 bNAbs enhance NK-cell activation and degranulation, culminating in ADCC mediated killing of HIV-1 infected cells in vitro. A better understanding of the exhaustion profile of NK-cells from PLWH will be necessary to address the potential of afucosylated antibodies for HIV-1 therapy.

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PP 8.9 – 00142

Safety and activity of BCL-2 inhibitor Venetoclax in uninfected rhesus macaques

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Background: In spite of the success of ART suppressing HIV replication, effective strategies to prevent the establishment and maintenance of the HIV reservoir are needed. Recent data indicate that BCL-2 contributes to the establishment, persistence and expansion of the reservoir; thus, there is a strong rationale behind promoting apoptosis of latently HIV-infected cells via BCL-2 inhibition. Herein, we evaluate in rhesus macaques (RMs) the pharmacokinetics, safety and efficacy of of Venetoclax, an FDA-approved BCL-2 inhibitor for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma.

Methods: 4 uninfected RM received 3 cycles of Venetoclax administered once daily for 4 consecutive days with a 2 week-interval between dosing cycles. RMs received Venetoclax as either an oral gavage (OG) (n = 2; 15 and 20 mg/kg), intramuscular injection (IM) (n = 2; 10, 15, and 20 mg/kg), or as an subcutaneous injection (SC) (20 mg/kg). Venetoclax was quantified in the plasma by liquid chromatography tandem mass spectrometry (LC-MS/MS). Flow cytometry analysis on peripheral blood mononuclear cells were performed to quantify the impact of Venetoclax on the frequency and absolute counts of lymphocytes and monocyte subsets and their levels of activation.

Results: Venetoclax was well tolerated for 4 consecutive doses. Venetoclax plasma concentration peaked 2 to 6 hours after administration, with the SC and IM routes resulting in similar peak, and higher than OG. Venetoclax administered through the IM and SC routes was effective in reducing the number of lymphocytes, including CD4+ T-cells expressing FOXP3 and T-bet, which expressed the highest levels of BCL-2 and constitute a critical component of the replication competent viral reservoir in ART-treated RMs. We also observed a preferential expansion of classical and intermediate monocytes, which express BCL-2 at levels significantly lower than non-classical monocytes that, in contrast, were reduced in frequency.

Conclusions: This proof of concept study identified a safe dose of Venetoclax able to depleting key cellular subsets of the HIV/SIV reservoir. We are now evaluating the activity of Venetoclax administration concomitantly with ART initiation in preventing the establishment and maintenance of the latent HIV reservoir.

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Evaluation of HIV-specific T cell response in BEAT2 clinical trial

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Background: Passive administration of HIV-1 broadly neutralizing antibodies (bNAbs) can achieve durable viral suppression when replacing ART. Previous studies have suggested that bNAb administration can have a vaccinal effect on HIV-specific T cells, increasing response frequencies and improving functional properties during bNAb-mediated suppression. Here, we evaluated whether such a vaccinal effect occurred in the BEAT2 clinical trial, which tested a 26-week combination of bNABs (3BNC117 and 10-1074) and peg-IFN-α2b (IMM-Tx) off-ART, followed by an off-IMM-Tx follow-up non-intervention ATI (non-int. ATI).

Methods: Peripheral mononuclear cells were obtained from the BEAT2 study (NCT03588715) in which baseline bNAb-sensitive PLWH received 29 weekly doses of peg-IFN-α2b (1.5 μg/kg) (4 wks on ART and 26 wks off ART), and seven IV infusions of the bNAbs (30 mg/kg during the 26 wks off ART). Ten participants received combined IMM-Tx, and one received only bNAbs infusions. Activation-induced marker (AIM) assay and flow cytometry were used to quantify HIV-specific immune responses in all 11 donors at baseline (on ART), after 4× weekly peg-IFN-α2b on ART, after 26 weeks of IMM-Tx (7 bNABs infusions plus peg-IFN-α2b), and during the final non-int. ATI. HIV1-specific CD4+ or CD8+ T cells were identified as CD69+PDL1+ or CD69+CD137+ or PDL1+CD137+ after stimulation against HIV-1 Consensus B Gag peptide pool.

Results: We found no increase in Gag-specific T cell responses during IMM-Tx (with or without peg-IFN-α2b) compared to

baseline in most donors; increased CD8+ responses after peg-IFN-α2b were observed in a single participant. We did find an increase in the proportion of Gag-specific CD4+ and CD8+ T cells in 4/11 individuals during the post-IMM-Tx non-int. ATI compared to earlier time points. These individuals showed sustained control of viremia (pVL 20 c/ml for 3 participants and <2000 c/ml for 1), suggesting an association between emerging T cell response and control of the viremia.

Conclusions: We found no detectable change in HIV-specific T cell responses after 26 weeks of IMM-Tx with bNABs plus peg-IFN-α2b immunotherapy; however, an increase in Gag-specific T cell responses was associated with viral control following the end of IMM-Tx in a subset of persons, suggesting a potential link between IMM-Tx and T cell-mediated responses in viral suppression.

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Distinct HIV reservoir characteristics among individuals treated during primary versus chronic HIV infection

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Background: To date, broadly neutralizing antibodies (bNAbs) have primarily been studied in people who initiated ART during chronic infection. Several cases of ART-free HIV remission ("post-treatment control") were observed following HIV immunotherapy with bNAbs. Individuals that started ART during primary HIV infection may have a lower barrier to achieving HIV remission. Here we characterize and compare the HIV reservoir size and composition between individuals treated during primary versus chronic HIV infection.

Methods: The ongoing RIO (Rockefeller-Imperial-Oxford, NCT04319367) clinical trial tests the combination of long-acting bNAbs (3BNC117-LS and 10-1074-LS) among individuals who started ART during primary HIV infection. To characterize the HIV reservoir in this primary infection cohort, we performed Q4PCR on baseline ("pre-bNAb therapy") samples from 18 RIO study participants who started ART within 6 months of confirmed primary HIV infection and had been on ART for a median of 67 (range 34–126) months. We compared the baseline proviral sequencing results obtained from RIO participants with a Q4PCR data set from 23 participants who started ART during chronic infection and had been on ART for a median of 84 (range 12–348) months (Gaebler et al.–Prolonged viral suppression with anti-HIV-1 antibody therapy. Nature 2022).

Results: Overall, we recovered 1360 defective and 67 intact proviral sequences from the RIO primary infection cohort. As expected, the overall reservoir size (Total proviruses per 106 CD4 + T cells) was significantly smaller in individuals treated during primary compared to chronic infection (Fig. 1a). Interestingly, the absolute number and relative representation of genetically intact proviruses among all sequences was significantly smaller following early initiation of ART (Fig. 1a, b). In addition, there were substantial differences in the absolute number of defective proviruses and the proviral composition of certain categories of defective proviruses in primary infection (Fig. 1a, b).

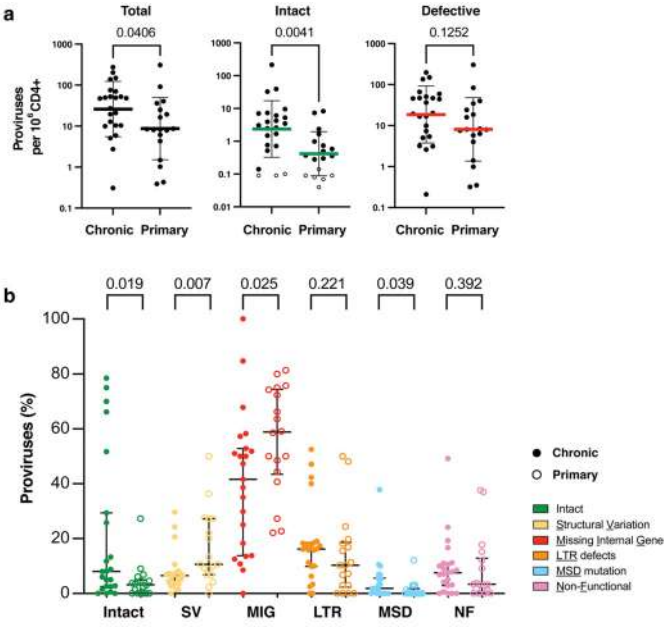

Figure 1. Reservoir quantification (a) and relative representation of proviral subtypes (b) in chronic (closed circle) or primary infection (open symbol).

Conclusions: We conclude that the proviral landscape differs in individuals who were treated early versus late after infection. These findings support the inclusion of detailed analyses of proviral reservoir size and composition to determine potential mechanisms and predictors of long-term ART-free HIV remission in the context of antibody-mediated therapy.

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In Vitro Assay for Escape Pathways from Broadly Neutralizing Antibodies

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Background: Broadly neutralizing antibodies (bNAbs) provide a useful tool for HIV cure strategies because of their ability to target conserved regions on the envelope (Env) protein in the context of both virions and infected cells. Multiple clinical trials infusing both mono- and combination bNAb therapies into people living with HIV demonstrated transient viral suppression after infusion. The major obstacle to more effective treatment, however, continues to be the ease of viral escape. In order to understand escape, we are determining common escape pathways from bNAbs to better inform future combination trials.

Methods: We designed an *in vitro* viral escape assay to test bNAb and Env combinations in a high throughput manner. We validated this assay with YU2 and bNAbs VRC01 and 10-1074. *Ex vivo* CD4+ T cells were infected in the presence of varying bNAb concentrations. Cultures were maintained for 60 days with suboptimal concentrations of bNAbs to induce escape.

Replication kinetics were monitored by p24 every 3 days. Every 14 days, target cells were replenished and cultures were tested for genotypic and phenotypic measures of bNAb resistance. This was accomplished by single genome sequencing *env* genes from the supernatant and using the remaining supernatant to spinoculate new CD4+ cells. The remaining infected cells were replenished with media containing no bNAb for 24 hours of virus production. This viral supernatant was then tested by neutralization assay for bNAb resistance.

Results: We observed both common escape mutations previously published, but also novel escape mutations. Partial resistance to VRC01 began at day 30 followed by complete resistance by day 45. At which point mutations were observed in the CD4 contact site as well as the fusion peptide in antibody-only wells. Complete resistance to 10-1074 developed by day 45 due to the N332 glycan mutation. A repeat independent experiment confirmed partial resistance developed to both antibodies by day 30.

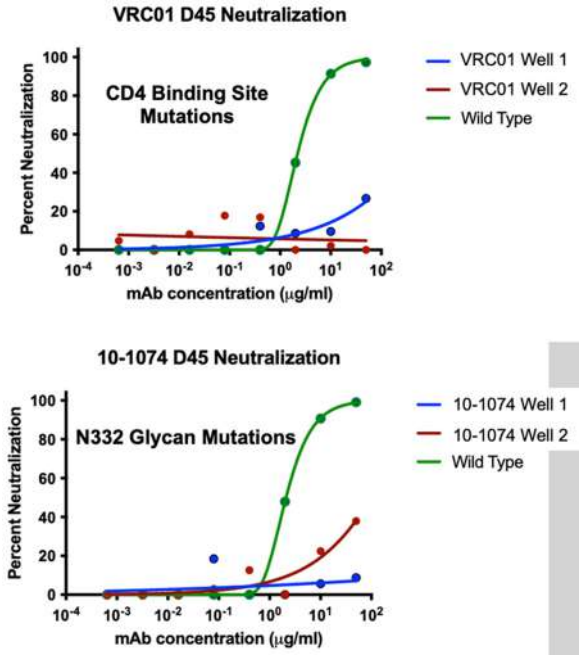

Figure. Antibody Resistance at Day 45.

Conclusions: Future experiments will expand viruses to multiple subtypes and bNAbs. This reproducible in vitro bNAb escape assay will lead to a deeper understanding of viral escape, to better inform the design of highly effective bNAb cocktails targeting multiple conserved sites.

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Investigating the Impact of CD4 mimetic BNM-III-170 on SHIV-infected Rhesus Macaques

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Background: Anti-HIV antibodies capable of mediating ADCC are elicited by the majority of people living with HIV and preferentially target the open, CD4-bound conformation of HIV-1 envelope glycoproteins (Env). However, due to the closed conformation of unliganded HIV-1 Envs, these antibodies are ineffective in ADCC-mediated clearance of infected cells. BNM-III-170 is a small molecule CD4 mimetic that binds to the closed conformation of HIV-1 Env, forces the envelope into an open, CD4-bound conformation, and exposes epitopes that are targeted by ADCC-mediating antibodies. Here, we evaluated the safety, pharmacokinetics, and virological impact of BNM-III-170 in viremic and ART-treated SHIV-AD8-EO-infected rhesus macaques (RMs).

Methods: Four BNM-III-170 dosing regimens were first evaluated in four chronically SHIV-AD8-EO-infected RMs. Plasma levels of BNM-III-170, biological activity of bioavailable BNM-III-170, plasma viral loads (PVLs), CBCs, and blood chemistries were measured. Following establishment of a well-tolerated BNM-III-170 dosing regimen, 12 RMs were intravenously inoculated with SHIV-AD8-EO (200 TCID50) and initiated antiretroviral therapy (ART) at 8 wks post infection (pi). At 34 wks pi, 6 animals began receiving 15 consecutive doses of 36 mg/kg BNM-III-170 subcutaneously, once every 3 days, while the other animals remained untreated. Analytical treatment interruption was performed at 37 wks pi and PVLs were monitored for 5 additional months.

Results: 36 mg/kg doses of BNM-III-170 administered every 3 days was determined to be safe in chronically SHIV-infected RMs. Importantly, bioavailable BNM-III-170 in the plasma of treated animals increased the binding of primary rhesus antibodies to infected cells and the plasma of treated animals supported ADCC in the presence of BNM-III-170. However, BNM-III-170 treatment did not change PVLs in the chronically infected RMs. Furthermore, in ART-treated RMs, no differences in viral

rebound, total SHIV DNA levels, or caRNA levels were observed between untreated and BNM-III-170-treated animals.

Conclusions: We established a safe and well-tolerated BNM-III-170 dosing regimen in chronically infected and ART-treated SHIV AD8-EO-infected RMs and demonstrated the biological activity of bioavailable BNM-III-170. However, we observed no impact on the viral reservoir with BNM-III-170 treatment. More potent CD4 mimetic compounds and increases in ADCC-mediating antibody levels may be needed to reduce the viral reservoir in SHIV-infected RMs.

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