Towards an HIV Cure Symposium 2014
Abstract Book

CONTENTS

Oral Abstract Session 1

OA1-1 1
OA1-2 2
OA1-3 3
OA1-4 4

Oral Abstract Session 2

OA2-1 5
OA2-2 7
OA2-3 8
OA2-4 LB 9

Oral Abstract Session 3

OA3-1 10
OA3-2 12
OA3-3 13
OA3-4 LB 14
OA3-5 LB 15

Oral Abstract Session 4

OA4-1 16
OA4-2 17
OA4-3 18
OA4-4 19
OA4-5 20
OA4-6 LB 21

Poster Exhibition

Innate Immune Responses and Function During HIV Infection 22
Virus-Specific Humoral Immunity 24
Virus-Specific Cellular Immunity 25
Immune Responses in Resistant Cohorts: Long-Term Non-Progressors and Highly Exposed, Seronegative Individuals 26
Correlates of Immune Protection 27
Mechanisms of Activation / Inflammation and Impact on Pathogenesis 28
HIV Replication and Pathogenesis in Other Organs and Tissues 32
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral Replication (Including Necessary Cellular Elements)</td>
<td>34</td>
</tr>
<tr>
<td>Viral Mechanisms of Persistence and Latency</td>
<td>35</td>
</tr>
<tr>
<td>Host Cellular Factors and Latency</td>
<td>40</td>
</tr>
<tr>
<td>Tissue Reservoirs</td>
<td>45</td>
</tr>
<tr>
<td>Measurement of Reservoirs</td>
<td>48</td>
</tr>
<tr>
<td>Targeting and Eradication of Reservoirs</td>
<td>51</td>
</tr>
<tr>
<td>Host Genetics of Resistance and Susceptibility</td>
<td>62</td>
</tr>
<tr>
<td>Host Restriction Factors (Including APOBEC and TRIM)</td>
<td>64</td>
</tr>
<tr>
<td>Therapeutic Vaccines</td>
<td>65</td>
</tr>
<tr>
<td>Animal Models of Latency Reservoirs and Eradication</td>
<td>69</td>
</tr>
<tr>
<td>Immune-Based Therapy Trials</td>
<td>70</td>
</tr>
<tr>
<td>Eradication / Reservoir Depletion: Impact of ART and Non-ART Treatment</td>
<td>72</td>
</tr>
<tr>
<td>Novel Therapeutic Approaches (Including Gene Therapy)</td>
<td>76</td>
</tr>
<tr>
<td>Mixed Methods, Integrated Approaches and Synergies in HIV Research</td>
<td>77</td>
</tr>
<tr>
<td>and Intervention</td>
<td></td>
</tr>
<tr>
<td>Community Engagement in Research and Research Dissemination</td>
<td>78</td>
</tr>
</tbody>
</table>
OA1-1

A population of CD8 T cells is located in germinal centers that is functionally capable of mediating bispecific antibody mediated killing of HIV-infected T-cells

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Background: T follicular helper CD4 T cells are located within the germinal centers (GC) of lymph nodes (LN). They are a source of residual viral replication during antiretroviral therapy and a contributor to the latent reservoir. Bispecific antibodies that target HIV Env and CD3 are being developed to purge the latent reservoir by activating HIV from CD4 T cells and inducing killing of those cells by CD8 T cells. We characterized the localization, frequency, and function of CD8 T cells in GCs to determine if they were present and capable of killing HIV-expressing cells in the context of HIV Env/CD3-targeting bispecific antibodies.

Methods: We analyzed the phenotype, localization and function of CD8 T cells in tonsils and LNs from non-infected and HIV-infected viremic individuals. Polychromatic flow cytometry was used for phenotypic analysis and confocal imaging for spacial localization. Function (IFNγ, TNF, MIP-1α, and GzB production) was assessed by intracellular staining after 5 hour anti-CD3 stimulation. in vitro cytolytic activity of sorted CD8 T cell populations was tested in a killing assay using an anti-HIV Env/anti-CD3 bispecific-antibody.

Results: Phenotypic analysis of tonsillar cells revealed a memory population of CD8 T cells expressing a CCR7lowCXCR5high profile compatible with follicular localization, while confocal imaging confirmed the presence of a small population of CD8 T cells within the GC. These GC CD8 T cells were expanded in HIV-infected LNs compared to non-infected tonsils. Functional activity in response to TCR stimulation occurred almost exclusively in CD8 T cells localized to the GC (defined by CXCR5 expression and loss of CCR7). Production of MIP-1α and GzB predominated over IFNγ and TNF production in GC CD8 T cells. Of all tissue CD8 T cell populations tested, GC localized CD8 T cells had the greatest ability to mediate killing of HIV-infected target cells after cross-linking with an anti-HIV Env/anti-CD3 bispecific antibody.

Conclusions: HIV infection is characterized by accumulation of CD8 T cells within LN follicles. These CD8 T cells are functionally capable of mediating bispecific antibody-mediated killing of HIV-infected CD4 T cells. These data add credence to the use of bispecific antibody therapy to purge the LN reservoir.
Potent and broadly anti-HIV-1 neutralizing antibody VRC01 inhibits HIV-1 transmission from plasmacytoid dendritic cells to CD4 T lymphocytes

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Background: Plasmacytoid dendritic cells (pDC) link innate and adaptive immunity and produce antiviral cytokines, particularly interferon-\(\alpha\) (IFN-\(\alpha\)). They express the host restriction factor SAMHD1, limiting HIV-1 infection of these cells. Interestingly, pDC have been described to transfer HIV-1 to CD4 T-lymphocytes. Here, we aim to analyze the ability of HIV-1 broadly neutralizing antibody (bNAb) VRC01 to inhibit HIV-1 transfer from pDC to primary autologous CD4 T-lymphocytes, and to investigate SAMHD1-mediated restriction in pDC.

Methods: Primary pDC were infected with R5 HIV-1 isolates and cocultivated with autologous activated CD4 T-lymphocytes in the presence or absence of bNAb VRC01. At 72h post-infection, the percentages of infected cells based on detection of intracellular viral p24 antigen were determined. We distinguished the pDC from the lymphocytes by specific membrane staining (CD123\(^+\)CD3\(^-\) for pDC, CD3\(^+\)CD123\(^-\) for CD4 T-lymphocytes) by flow cytometry. In parallel, the expression of intracellular SAMHD1 and CD83\(^+\)/CD86\(^+\)CD123\(^+\) maturation markers were quantified in pDC. IFN-\(\alpha\) production was measured in the supernatant of cocultures. Virus-like particles containing Vpx (VLP-Vpx) was used to decrease SAMHD1 restriction.

Results: We found that pDC efficiently transferred HIV-1 in trans and in cis to adjacent CD4 T-lymphocytes via cell-to-cell transfer. Interestingly, coculture with CD4 T-lymphocytes downregulated SAMHD1 expression, enhanced HIV-1 replication, induced pDC maturation and increased IFN-\(\alpha\) secretion. BNAb VRC01 added on HIV-1-loaded pDC was able to block trans- and cis-transfer with a similar efficiency as cell-free infection of CD4 T-lymphocytes.

Conclusions: Our results demonstrate that, in a physiologically relevant model of pDC/lymphocyte coculture, autologous lymphocytes downregulate SAMHD1 expression in pDC, triggering HIV-1 replication and an antiviral immune response. BNAb VRC01 efficiently inhibits HIV-1 transfer from pDC to CD4 T-lymphocytes. These results suggest that at mucosal portal of HIV-1 entry, the enhanced virus replication in pDC may promote HIV transfer to neighboring CD4 T-lymphocytes, but HIV-1 specific antibodies can prevent this early virus dissemination following sexual transmission. This work was supported by ANRS and EuroNeut41 grants. Dr. J. R. Mascola kindly provided bNAb VRC01 (NIH) and Dr. O. Schwartz kindly provided Ab against human SAMHD1 and VLP-Vpx (Institut Pasteur).
HIV Controller CD4+ T cells preferentially express a public TCR clonotype that confers high avidity responses against Gag

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Background: HIV Controllers are rare patients who spontaneously control HIV replication to levels below 50 copies viral RNA/ml in the absence of antiretroviral therapy. We previously reported that HIV Controllers harbor a pool of memory CD4+ T cells able to respond to minimal amounts of virus, due to the expression of T cell receptors (TCRs) with high avidity for immunodominant Gag epitopes. We set to characterize these high avidity TCRs at the molecular and functional levels.

Methods: HIV Controllers from the ANRS CO21 CODEX cohort (n=8) were compared to efficiently treated patients (n=8). Primary CD4+ T cell lines were generated by stimulating PBMCs with decreasing doses of the immunodominant Gag293 peptide. Specific CD4+ T cells were labeled with Gag293-loaded MHC class II tetramers and sorted. The TCR repertoire of tetramer+ cells was evaluated by CDR3 length polymorphism analysis (Immunoscope) and sequencing. Highly represented TCR Vα and Vβ chains were cloned in bicistronic lentiviral vectors and tested for CD69 induction and cytokine production.

Results: Stimulation with low Gag293 peptide doses generated IFNγ-positive CD4+ T cell lines for HIV Controllers, but rarely for treated patients, confirming the predominance of high avidity cells in the Controller group. Immunoscope analysis revealed a major amplification of TCR Vα24 chains with a 10 a.a. CDR3 in sorted tetramer+ cells from Controllers, while this amplification was rarely detected in treated patients (P<0.05). Sequencing revealed the presence of a public TCR Vα24-J17 clonotype shared by 6 Controllers and 2 treated patients. TCRs comprised of the public Vα24 chain and of highly expressed Vβ chains conferred high avidity Gag293 recognition and polyfunctionality to transduced T cells. One of these TCRs could achieve Gag293 recognition in the context of 4 distinct HLA-DR molecules, possibly explaining its public nature.

Conclusions: We identified a highly prevalent TCR sequence preferentially expressed by Gag-specific CD4+ T cells from HIV Controllers. This public clonotype confers high avidity polyfunctional responses to Gag, raising the possibility that it could be used as molecular marker of efficient responses against HIV.
Co-localization of follicular SIV-specific CD8 T Cells with Tfh in the germinal centers of chronically SIV infected rhesus macaques is associated with enhanced viral control

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Background: Chronic SIV/HIV infection promotes an accumulation of virus-infected T follicular helper cells (Tfh cells, PD-1 hi, CXCR5+, Bcl6+) in the lymph nodes (LN), an important site of viral replication but the fate of these cells in the rectal mucosa (rectum), another preferential site of virus replication, is not known. Furthermore, SIV specific CD8 T cells, indispensible for viral control and extensively studied in the periphery, are less understood at follicular sites. Here we studied the fate of PD-1 hi CD4 T cells in the rectum and LN during chronic SIV infection in a cohort of unvaccinated and DNA/MVA vaccinated rhesus macaques (RM) and the importance of follicular CD8 T cells at these sites.

Methods: Lymphocytes isolated from LN and rectum of SIV naïve and SIV infected RM were separated and characterized by multi-color flow cytometry. Sorted cells were used for measuring cell associated viral RNA by qRT-PCR. Cellular localization was determined by immunofluorescence staining.

Results: Following a pathogenic SIV infection, despite a global depletion of CD4 T cells, Tfh cells increased dramatically in animals that failed to control SIV infection (set point >10^4 viral RNA copies/ml of plasma). Moreover, these PD-1 hi CD4 T cells were enriched in viral RNA, indicating that they actively support viral production. In contrast, vaccine-mediated viral control (plasma viral loads below 10^4 viral RNA copies/ml) was associated with limited Tfh expansion at these lymphoid sites. Interestingly, higher frequencies of functional SIV-specific CD8 T cells (CD8+ Granzyme B+ CXCR5+ CM9-Tet+) correlated with decreased Tfh frequencies and improved viral control in the LN follicles. Further investigation, using immunofluorescence staining, of both rectal and LN tissues from vaccine controllers revealed colocalization of CD8 T cells with PD-1 bright cells in IgD- GC, a phenomena strikingly not observed in the SIV infected non-controlling RM.

Conclusions: These data demonstrate that SIV-infected Tfh cells accumulate in GC of the LN and rectum during uncontrolled chronic SIV infection evading anti-viral CD8 T cell responses. They suggest a possible mechanism by which infiltration of functional SIV-specific CD8 T cells to GC of lymphoid sites limits Tfh expansion and contributes to enhanced viral control.
Synergistic activation of HIV-1 expression by compounds releasing active positive transcription elongation factor b (P-TEFb) and by inducers of the NF-kB signaling pathway

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Background: Despite cART, the persistence of cellular reservoirs harboring transcriptionally silent but replication-competent HIV-1 proviruses is a major hurdle to virus eradication. Various strategies to purge these reservoirs are therefore under intense investigations. Bromodomain and Extraterminal (BET) inhibitors (JQ1, I-BET, I-BET151) alone or in combination with Prostratin have recently been identified as compounds able to reactivate HIV-1 from latency. This activation is dependent on the positive transcription elongation factor b (P-TEFb). Moreover, the dependency on Tat viral protein was investigated and is still controversial.

Here, we investigated the reactivation potential of compounds releasing active P-TEF-b (HMBA, JQ1, I-BET, I-BET151) alone or in combination with two NF-kB inducers (Prostratin and Bryostatin-1) in vitro on several latency model cell lines and ex vivo on cART-treated patients primary cells.

Methods: p24 ELISA assays, RT-qPCR, FACS, cytotoxicity tests, transient transfection and chromatin immunoprecipitation assays. The reactivation tests were carried out in T-cell, monocytic or microglial cell lines and in ex vivo cultures of CD8⁺-depleted PBMCs or resting memory CD4⁺ T cells isolated from HIV-1⁺ cART-treated aviremic patients.

Results: We found that NF-kB inducers and compounds releasing P-TEFb increased HIV-1 production in a dose-dependent manner, with minimal cytotoxicity, in several postintegration latency model cell lines, at both the viral mRNA and protein levels. Next, we observed that co-treatment led to strong synergistic activation of HIV-1 production in these models. FACS analysis revealed that the combinatory treatments increased HIV-1 expression in a higher proportion of cells than the compounds alone.
We demonstrated that the NF-kB binding sites located in the HIV-1 5’LTR enhancer were required for this synergistic activation. This synergistic effect was independent on the Tat/TAR axis.

Importantly, we demonstrated the physiological relevance of our data in ex-vivo cultures of CD8⁺-depleted PBMCs or resting memory CD4⁺ T cells isolated from aviremic HIV-1⁺ cART-treated patients, by showing that combinatory treatments exhibited a higher reactivation potential that the compounds alone.

**Conclusions:** Our results suggest the administration of compounds releasing active P-TEFb combined with PKC activators together with continuous cART as a potential strategy to reactivate HIV-1 from latency.
A new family of compounds that reactivate latent HIV in central memory T-cells

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**Background:** Reactivation of latent HIV in vivo for the purpose of purging the viral reservoir has become a formidable challenge. One major problem is to find stimuli that will effectively reactivate the virus without causing overt T-cell activation and proliferation. To address this problem, we have performed a medium-throughput screen of chemical and natural product libraries. This work describes the discovery, the properties and the chemical structure of a compound (hereby referred to as “C7”) that was found through this screen.

**Methods:** We have used a high-throughput variation of a published assay of viral latency in central memory T cells, and identified a novel family of compounds that reactivate latent HIV-1. The biological properties of these compounds were further investigated in order to examine their ability to induce activation markers (CD25 and CD69) and proliferation. This was done in parallel with antigenic stimulation as a comparison.

**Results:** We have identified a family of compounds (“C7”) which, when incubated with latently infected T\(_{\text{CM}}\) cells, display viral reactivation ability that is about 70-80% of that obtained with anti-CD3/CD28 stimulation. The activation profile of the C7-treated cells was indistinguishable from that of untreated, resting cells as evidenced by a lack of induction of CD25 and CD69. C7 also failed to induce cellular proliferation in the range of concentrations tested. C7 activates latent HIV through sustained phosphorylation of STAT5, which contrasts with the transient phosphorylation of STAT5 that is observed with gamma-c cytokine stimulation. C7 induced reactivation from latency can be blocked by JAK and STAT5 inhibitors. Surprisingly, stimulation of cells with C7 reactivated latent HIV without inducing a concomitant increase in cyclin T levels or CDK9 phosphorylation. When tested in the virus outgrowth assay that uses aviremic patient cells, C7 was able to reactivate latent viruses.

**Conclusions:** These results demonstrate that a JAK/STAT-mediated signaling pathway can be utilized to trigger activation of latent proviruses in primary cells in vitro. Key signaling elements controlling this pathway should be considered as novel targets. The C7 compound and related molecules targeting this pathway belong to a chemical family not previously used as pharmacological agents.
Induction and clearance of latent HIV infection: modeling viral clearance by immune effectors using cells from ART-treated patients


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**Background:** An effective immune response is necessary to eliminate reactivated latent reservoirs. Ex-vivo expansion of HIV specific T cell lymphocytes (HXTCs) derived from HIV infected patients may enhance the immune response. In addition, cytokine-stimulated Natural Killer (NK) cells may effectively clear HIV-infected cells.

**Methods:** Enhanced HXTCs were established from the cells of HIV-infected patients on ART by stimulation with autologous dendritic cells and PHA lymphoblasts loaded with overlapping peptides spanning consensus regions of HIV-1 gag p24, pol, and nef. CD56+ NK cells were isolated by negative selection and stimulated for 24-hours with 100U IL-2/mL. Viral inhibition activity was assessed in superinfection assays using JR-CSF virus or autologous reservoir virus (virus recovered from the same patient’s resting CD4+ cells) in the presence or absence of either HXTCs or NKs. A novel latency clearance assay was devised, in which resting CD4 cells were reactivated and co-cultured with HXTCs or NK cells.

**Results:** HXTCs reduced viral production after superinfection with JR-CSF (median %p24 produced with HXTCs=2.5%, vs 29.2% with unexpanded CD8s, p< .05) or autologous reservoir virus (median 8% vs. 20.6%, p< .05). Stimulated NKs reduced viral production to 10.58%(p< .05), 53.57%(p< .05) and 84.90%(p=0.35) at 1:1, 1:10 and 1:100 effector:target ratios. In latency clearance assays, HXTCs showed a significant reduction in virus recovered from reactivated resting CD4 cells (median 50% virus recovered, p=.03), superior to that seen with unexpanded autologous CD8 cells (median 90% virus recovered). Unstimulated NKs reduced viral recovery to 54%, and stimulated NKs reduced this further to 28.6% recovery. In preliminary studies, antiviral activity is also seen when latency is disrupted by HDAC inhibitors.

**Conclusions:** Both ex-vivo expansion of T cells and cytokine stimulation of NKs showed improved in-vitro and ex-vivo anti-HIV activity, including an enhanced ability to clear latent HIV infection. Ex-vivo expanded T cells and NK cells could thus prove useful in combination with latency reactivating agents in future studies.
The HDAC Inhibitor Romidepsin is Safe and Effectively Reverses HIV-1 latency in vivo as Measured by Standard Clinical Assays

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Background: In a recently published ex vivo study, the latency reversing agent (LRA) romidepsin induced HIV expression in resting CD4+ T cells isolated from patients undergoing combination antiretroviral treatment (cART). In light of this exciting finding, we evaluated the effects of romidepsin on measures of viral transcription and plasma viremia in vivo.

Methods: In a phase I/II clinical trial, six aviremic HIV-infected adults received intravenous romidepsin (5 mg/m²) once weekly for 3 weeks while maintaining cART. We used flow cytometry to determine H3 histone acetylation levels in lymphocytes as a cellular measure of the pharmacodynamic response to romidepsin. Changes in intracellular viral transcription were quantitated by cell-associated unspliced HIV-1-RNA (CA-US HIV-1-RNA) using digital droplet PCR in unfractionated CD4+ T cells. Plasma HIV-1-RNA was analyzed by a standard clinical viral load assay (Cobas Taqman) and a transcription-mediated amplification (TMA) assay (Procleix Ultro Plus). Safety was evaluated at each study visit. Baseline values were compared with post-infusion values using Wilcoxon signed-rank tests. Binary outcomes were analyzed using two-sided binomial exact tests.

Results: All 6 patients (5 males, 1 female) completed three romidepsin infusions. H3 histone acetylation increased rapidly (max 17.7 fold relative to baseline) within the first hours following each romidepsin administration and then decreased between day 3 and 7 day post-infusion. Concurrently, CA-US HIV-1-RNA levels increased significantly from baseline during treatment (2.1-3.9 fold after 2nd infusion; p=0.03). Importantly, viral load increased from “undetectable” at baseline to readily quantifiable levels at multiple post-infusion timepoints in 5 of 6 patients (range 46-103 copies/mL after 2nd infusion, p=0.007). Plasma HIV-1-RNA was also detected by TMA more frequently at post-infusion timepoints vs. baseline (p=0.03 after 2nd infusion). Furthermore, the emergence of quantifiable plasma HIV-1-RNA corresponded directly with the cyclic romidepsin infusions. Adverse events (all grade 1-2) were consistent with the known side effects of romidepsin and HDAC inhibitors in general.

Conclusions: Romidepsin safely induced HIV-1 transcription resulting in plasma viremia that was readily quantified with standard commercial assays. Our data show that potent in vivo latency reversal is possible with a single LRA. A trial combining romidepsin and therapeutic vaccination is ongoing.
OA3-1

Genetically characterizing the role of cell proliferation in maintaining persistent HIV during effective HIV therapy

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Background: The contribution of homeostatic cell proliferation to the size and maintenance of the latent HIV-1 reservoir is currently unknown. To address this issue, we examined the distribution of identical HIV-1 intracellular sequences from peripheral blood, GALT and lymph node tissue in order to define the role of homeostatic cell proliferation as a cause of persistence in different memory T cell subsets during long-term suppressive therapy.

Methods: Using single-proviral sequencing we isolated intracellular HIV-1 genomes derived from defined subsets of CD4+ T cells (naïve, central (CM)-, transitional (TM)-, and effector (EM)-memory) from peripheral blood, GALT and lymph node tissue (LNT). Samples were collected at two time points 6 months apart from 3 subjects on suppressive therapy (5-10 years) who initiated therapy during chronic infection. Unique clonal populations of sequences were determined (for p6-RT) as >2 genetically identical sequences among all the viruses analyzed for each subject (N=190-287) and compared to pre-therapy plasma-derived single genomes.

Results: Phylogenetic analyses showed expansions of identical HIV-1 sequences in all subjects: 30-50% of all intracellular sequences from both time points were members of 9-27 expansions of identical sequences. However, only three of these sequence expansions were observed in pre-therapy specimens. At both times points, clonal populations were found predominantly in EM cells from peripheral blood and LNT. At time point 2, the odds of a viral sequence being a clone in EM cells were 9-21 and 4-87 fold higher than if the viral sequence came from CM cells (p=0.0024-0.0001) and TM cells (p=0.016-<0.0001) respectively. In GALT, for time point 2, all identical sequences were found in EM cells but the number of cells analyzed from this tissue at both time points were limited. A large expansion of identical sequences in EM cells from the peripheral blood of one patient was found to be replication defective.
Conclusions: A large percentage of intracellular sequences, not detected in pre-therapy plasma, are clonal in nature and enriched in more differentiated cells, suggesting that HIV persistence during effective therapy is driven in large part by the proliferation, differentiation and expansion of cell populations with sustained and durable regenerative potential.
TLR2 stimulation promotes HIV-1 infection of CD4+ T-cells by increasing the susceptibility of CCR6- T-cells to infection

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Background: Following infection, HIV-1 induces an immunological and structural disruption of the gut mucosa, leading to bacterial translocation and release of microbial components in the bloodstream. Those pathogen-associated molecular pattern acting as toll-like receptor (TLR) agonists may affect gut-homing CD4+ T cells (T4 cells), such as CCR6+ T4 cells, which are highly permissive to HIV-1 infection. In this study, we investigated the effect of TLR2 ligation on the susceptibility of CCR6+/- T4 cells to HIV-1 infection.

Methods: Primary human resting T4 cells were purified by magnetic separation, stimulated for 72 h with anti-CD3/CD28 antibodies +/- Pam3CSK4 (TLR2 ligand) and infected for 24h with the HIV-1-based reporter virus NL4-3-Bal-IRES-HSA. To investigate the effect of TLR2 ligation on T4 cells’ susceptibility to HIV-1 infection, the cell percentage expressing HSA surface protein, as well as cell proliferation, were analyzed by flow cytometry, the modulation of infectability was confirmed by ELISA p24 and the HIV-1 integration was evaluated by qPCR. To examine the effects of TLR2 ligation on the relative susceptibility of CCR6+/- T4 cells, the percentage of subset-specific HSA+ cells and CD4/CCR5/α4 surface expression were analyzed by flow cytometry.

Results: Compared to CD3/CD28 stimulation, TLR2 ligation induced a 3-fold increase in the percentage of infected (HSA+) T4 cells (n=7) and viral p24 production (n=5). Furthermore, TLR2 ligation induced a 1.5-fold increase in the HIV-1 DNA integration (n=4) without affecting cell proliferation (n=5). TLR2 ligation increased the susceptibility of CCR6- cells to HIV-1 infection to levels observed in the CCR6+ subset, while CD3/CD28 stimulation by itself increased infection of CCR6+ cells (n=5). CD4/CCR5/α4 cell surface expression was higher on CCR6+ than on CCR6- T4 cells, but was not affected by TLR2 ligation (n=3).

Conclusions: Our results suggest that TLR2 stimulation promotes early HIV-1 infection of T4 cells by abolishing barriers acting at both pre- and post-integration steps. We also propose that the effect of TLR2 ligation on HIV-1 infection susceptibility is more pronounced on CCR6- than on CCR6+ T4 cells. These results highlight the importance of early interactions between HIV-1 and gut microbiota, which could lead to novel therapies seeking to block those interactions.
Interleukin-21 improves reconstitution of intestinal Th17 and Th22 cells and reduces residual inflammation in ART-suppressed SIV-infected rhesus macaques

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Background: Current HIV antiretroviral therapy (ART) inhibits virus replication and prolongs life expectancy in people living with HIV. Although HIV replication is suppressed, residual inflammation persists and critically contributes to non-AIDS-related morbidity and mortality in ART-treated individuals. Interleukin (IL)-21 regulates the differentiation and maintenance of IL-17- and IL-22-producing CD4 T cells, which depletion critically contributes to chronic immune activation and disease progression in HIV infection. Previously, we showed that when administered during acute infection IL-21 reduces microbial translocation and systemic immune activation in SIV-infected rhesus macaques (RMs). Here, we investigated if administration of IL-21 to chronically SIV-infected, ART-treated RMs is effective in limiting the extent of residual inflammation.

Methods: Sixteen RMs were infected with SIVmac239 i.v. and, starting at day 60 post-infection, treated for seven months with PMPA, FTC, Raltegravir, Darunavir and Ritonavir. Eight RMs received IL-21-Fc (100 mg/kg, s.c., weekly, six weeks) at the beginning and the end of ART, with the other eight serving as controls. Blood, lymph nodes and rectum were longitudinally collected, and the effects of IL-21 on inflammation, T cell subset levels and functions assessed by flow cytometry. The Mann-Whitney test was used for statistical analyses.

Results: ART was very effective, with fully suppressed plasma viremia (< 60 SIV-RNA copies/ml) in all RMs. Compared to ART-controls, ART+IL-21 RMs showed improved restoration of intestinal IL-17⁺, IL-22⁺, and IL-17⁺IL-22⁺ CD4 T cells (P < 0.01 for all subsets). Furthermore, the ability of these cells to produce multiple cytokines, as assessed by co-staining of IL-17, IL-2, TNF-α, and IFN-γ, was restored at levels significantly higher in IL-21-treated RMs than in controls. Remarkably, ART+IL-21-treated RMs showed higher reduction (P < 0.01) in the levels of activated (HLA-DR⁺CD38⁺), proliferating (Ki-67⁺) and PD-1⁺ T cells in rectum and blood when compared to ART-treated RMs. If the ability of IL-21 to reduce residual inflammation impacted on the size of the SIV reservoir is under investigation.

Conclusions: IL-21 administration in ART-suppressed SIV-infected RMs more rapidly restores intestinal IL-17 and IL-22 producing CD4 T cells while limiting residual inflammation. Thus, IL-21 may provide important therapeutic benefits when used as an immunomodulator in ART-suppressed HIV-infected individuals.
Cryptic transcription of HIV-RNA species from “defective” proviruses: A novel pathway for persistent immune activation in patients with HIV-1 infection and mechanism for persistent seropositivity despite “undetectable” levels of virus

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**Background:** Despite years of “successful” therapy, defined as HIV-RNA levels < 40 copies/ml, the majority of HIV-infected patients exhibit persistent seropositivity to HIV-1 and evidence of ongoing immune activation. They also have persistence of proviral DNA in circulating PBMCs although much of this proviral DNA has been characterized as “defective”. One setting in which this is not the case has been following bone marrow transplantation in which seronegativity has been reported in association with a loss of peripheral blood proviral DNA. The purpose of the present study was to better define the characteristics of the peripheral blood pool of proviral DNA in an effort to see whether or not “defective” proviruses are transcribed and capable of encoding viral proteins even if unable to encode an intact virus.

**Methods:** HIV-DNA/RNA were simultaneously purified from CD4+ T cells and subjected to single-molecule amplification using PCR primers that bound to the two LTRs. The expected sizes of PCR products with these primers are 9kb for HIV-DNA and 8.4 kb for HIV-RNA. Western blots were performed using the Cambridge Biotech HIV-1 Western blot kit. Sequence analyses were performed by the MiSeq, Sanger and/or PacBio systems.

**Results:** Proviral DNA and transcribed RNA were amplified from CD4+ T cells from seven HIV-infected patients on suppressive cART for >4 yrs. Six percent of proviruses were of 9kb. Of the remaining proviruses, 82% were 2-8 kb in length and contained 1-7 open reading frames capable of encoding Gag, Pol and Env primary proteins. A correlation was noted between incomplete banding profiles on serum western blots, presence of “defective” proviruses and cryptic transcription of novel HIV-RNA species.

**Conclusions:** These data suggest that some “defective” proviruses are capable of encoding viral proteins in the absence of intact viruses. The production of these proteins may explain the persistent seropositivity to HIV and chronic immune activation seen in patients despite years of suppressive cART. We propose the term “zombie proviruses” to refer to these species insofar as they are not “alive”, resemble intact proviruses, and are able to inflict harm. Strategies directed toward “curing” HIV need to include approaches designed to eliminate cells harboring such proviruses.
OA3-5 LB

IL-7 increases HIV-1 proviral integration in CD4+ T cells by inducing SAMHD1 phosphorylation, which can be blocked by Lck and PKCθ inhibitors

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Background: The establishment of HIV-1 post-integration latency in quiescent CD4+ T cells is not fully understood, but some cytokines and chemokines may facilitate it by inducing silent proviral integration. Homeostatic stimuli such as IL-7 and IL-2 would produce latent infection but not full replication. We hypothesized that this mechanism could be related to SAMHD1, an antiviral factor which activity is greatly dependent on cell cycle progression, as cyclin A2 and CDK1 are responsible for SAMHD1 phosphorylation (pSAMHD1) and subsequent inactivation.

Methods: Resting CD4+ T cells were obtained by negative selection from PBMCs isolated from healthy donors. pSAMHD1 at T592 was determined by immunoblotting. Retrotranscription (RT) and proviral integration was analyzed by qPCR using TaqMan probes.

Results: 1) IL-2 (300U/ml), IL-7 (1nM) and anti-CD3/CD28 induced pSAMHD1 at T592. This phosphorylated state was quite stable after the stimuli removal. 3) IL-7 did not induce T-cell proliferation, uncoupling T-cell division and pSAMHD1. 4) Treatment with chemokines (CXCL9, CXCL10, CXCL12) or TNFα did not induce pSAMHD1. 5) Expression of total SAMHD1 was not modified by any stimulus. 6) IL-2 or IL-7 alone resulted in full RT and proviral integration but viral transcription was barely detected and only increased after PMA treatment, proving the role in latency mostly of IL-7. 7) IL-7 and IL-2 induced low NF-κB and LTR-dependent viral transcription that was fully induced by PMA. 8) Specific Lck and PKCθ inhibitors interfered with pSAMHD1 induced by IL-7 or IL-2, blocking HIV-1 cycle from RT.

Conclusions: Homeostatic stimuli as IL-7 and IL-2 improved HIV-1 early steps -RT and proviral integration- in CD4+ T lymphocytes through pSAMHD1, improved by simultaneous treatment with CXCL9/CXCL10. We propose a three-step model: 1) homeostatic proliferation would allow full RT through pSAMHD1 and proviral integration, leading to the establishment of latent reservoirs. 2) Once RT is produced, chemokines would strongly enhance viral integration. 3) NF-κB/NF-AT induction by a third stimulus would drive HIV-1 transcription and full replication. In this model, SAMHD1 regulation plays a central role in the establishment of viral reservoirs and represents a major target for therapeutic intervention. Drugs able to interfere with pSAMHD1, thereby preserving its antiviral function, have been tested.
The immune checkpoint blockers PD-1, LAG-3 and TIGIT are biomarkers of HIV infected cells during ART and identify distinct cellular reservoirs

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Background: The persistence of HIV in a small pool of long-lived latently infected resting CD4+ T cells is a major barrier to viral eradication. Identifying cellular markers that are preferentially expressed at the surface of latently infected cells may lead to novel therapeutic strategies to cure HIV infection. We have previously shown that HIV primarily persists in central (TCM), transitional (TTM) and effector memory (TEM) CD4+ T cells and that the levels of expression of the immune checkpoint blockers (ICBs) PD-1, LAG-3 and TIGIT are associated with HIV persistence during ART. We hypothesize that these markers identify memory CD4+ T cells highly enriched in latent HIV by continuously promoting HIV latency in infected CD4+ T cells.

Methods: 20 subjects on ART for >3 years with HIV viral load < 50 cop./mL and with CD4 count >350 cells/µL enrolled in the study. We measured integrated HIV DNA in sorted memory CD4+ T cell subsets expressing PD-1, LAG-3, TIGIT or co-expressing the 3 receptors. Wilcoxon test was performed to compare the frequencies of cells harboring integrated HIV DNA in the ICBs expressing cells with their negative counterpart.

Results: PD-1 identified TCM and TTM cells enriched for integrated HIV DNA (p=0.019, p=0.004 respectively). Latently infected TEM cells tended to express TIGIT (p=0.078), but did not differ from uninfected cells in PD-1 expression. LAG-3 identified latently infected memory CD4+ T cells independently of their differentiation fate (p=0.002 for the combined memory subsets). Importantly, CD4+ T cells co-expressing PD-1, LAG-3 and TIGIT were highly enriched for integrated HIV DNA when compared to unsorted CD4+ T cells (4-10 fold increase).

Conclusions: Our data demonstrate that PD-1, LAG-3 and TIGIT identify cells carrying integrated HIV DNA in virally suppressed subjects. Our results further suggest that PD-1, LAG-3 and TIGIT specifically contribute to viral persistence during ART in distinct memory CD4+ T cell subsets. Novel curative strategies interfering with ICBs pathway may be differentially beneficial to ART treated subjects depending on the localization of their HIV reservoir.
**OA4-2**

**Following in vitro culture with myeloid dendritic cells, negative regulators of T-cell activation are expressed preferentially on latently infected CD4+ T-cells**

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**Background:** Long-lived latently infected resting CD4\(^+\) T-cells pose a major barrier to HIV cure. Latency can be established via direct infection of resting CD4\(^+\) T-cells, which is significantly enhanced by co-culture with myeloid dendritic cells (mDC). We hypothesised that the expression of negative regulators of T-cell activation during mDC-T-cell interactions may actively suppress viral replication leading to the establishment and maintenance of latency.

**Methods:** Resting CD4\(^+\) T-cells, isolated from the blood of healthy donors and labelled with the proliferation dye eFluor670, were cultured alone or with autologous blood mDC. Following 24h of culture, cells were infected with a CCR5-using EGFP-reporter virus. Expression of the negative regulators PD-1, CTLA-4 and Tim-3, and their corresponding ligands PDL-1/PDL-2, CD80/CD86 and Galectin 9, were measured on the resting CD4\(^+\) T-cells and mDC respectively, both at baseline and daily for 5 days post infection. Non-proliferating (eFluor\(^{high}\)), non-productively infected (EGFP\(^{-}\)) CD4\(^+\) T-cells were sorted by flow cytometry on day 5 post infection. Cells were then further sorted into populations expressing high or low/- levels of PD-1 or Tim-3. Sorted cells were cultured for 3 days in the presence of IL-7 and the integrase inhibitor L8 with or without anti-CD3/CD28 stimulation to quantify post-integration latent infection.

**Results:** Co-culture of resting CD4\(^+\) T-cells with mDC resulted in an increase in the expression of both PD-1 (mean fold change (MFC) =32, n=3) and Tim-3 (MFC=24, n=3), but not CTLA-4, when compared to resting CD4\(^+\) T-cells cultured alone. Ligands for all three negative regulators were expressed on the mDC at day 1 post infection (PDL-1 23%; PDL-2 4%; GaL9 46%; CD80 26%; CD86 62% of mDC). Post-integration latency was significantly enriched in sorted eFluor\(^{high}\)EGFP - CD4\(^+\) T-cells that expressed high levels of either PD-1 (MFC=4, p=0.04, n=5) or Tim-3 (MFC=4, p=0.04, n=4) when compared to T-cells that had little to no expression of the negative regulators.

**Conclusions:** Negative regulators of T-cell activation, PD-1 and Tim-3, were preferentially expressed on resting CD4\(^+\) T-cells that were latently infected following co-culture with mDC in vitro. Disrupting the function of these negative regulators could be a potential future strategy to reverse the establishment and/or maintenance of latency.
Ultra-deep barcoded SIVmac239 to identify and quantify viral reservoirs and recrudescent viremia

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Background: A primary obstacle in curing HIV infection with current antiretroviral therapies is the early establishment of long-lived viral reservoirs comprised of cells or anatomic sites that retain replication competent virus that persists for years even in the face of apparently suppressive antiretroviral treatment and can contribute to recrudescent viremia when treatment is stopped. There are major gaps in our understanding of viral reservoir establishment and maintenance that are particularly difficult to study in humans but can be studied in nonhuman primates (NHP). Here we report a novel molecular tag of SIV that can be used as a NHP model to identify the tissues and cellular sources of persistent SIV/HIV.

Methods: SIVmac239 was genetically engineered to include a molecular cassette between the vpx and vpr genes. The cassette allows for the insertion of 10 randomly generated oligonucleotides producing up to 1 million unique plasmid clones. Infectious virus is generated by transfection, producing a viral stock that is genetically identical except for the insertion. Barcoded viruses were examined in vitro (SupT1-cells) and in vivo (rhesus macaque) for replication competence and retention of barcode over time. Real-time single genome amplification and next generation sequencing was used to quantify the number of unique viral genomes.

Results: We have generated and validated this unique barcoding approach for use in vitro and in nonhuman primate models. We found no evidence of reduced replication competency, nor was the barcode lost or altered during culture or in vivo. Viral replication in rhesus macaques following intravenous inoculation revealed a very rapid and consistent viral load curves with peak viral loads at ~10^8 cp/ml with no differences noted from historic controls. Viral sequence analysis revealed a minimum of 1,000 viral variants in the viral inoculum.

Conclusions: We have created a novel reagent to track the establishment and persistence of persistent viral reservoirs and the sources contributing to recrudescent viremia following release of suppressive antiretroviral therapy.
In vivo analysis of HIV replication and persistence in the myeloid compartment

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Background: Latently infected CD4+ T cells and macrophages are cellular HIV reservoirs. The importance of monocyte/macrophages in HIV eradication is highlighted by the fact that they are major contributors to the entry of HIV into the CNS, a key anatomical reservoir of HIV. In order to elucidate the contribution of myeloid cells to HIV persistence, we have created a novel humanized myeloid only mouse model (MoM) where HIV systemic infection and persistence in myeloid cells can be studied in vivo.

Methods: MoM were created by transplanting human CD34+ hematopoietic stem cells into immunodeficient mice. Human-specific antibodies against CD45, CD3, CD19, CD33, CD14, CD16, and HLA-DR were used to characterize immune cell populations in the peripheral blood and tissues of MoM. To establish the susceptibility of MoM to HIV-1 infection, a variety of R5-tropic viruses were injected intravenously and plasma viral load was monitored longitudinally. Viruses included a replication-competent molecular clone of CH040 containing a CNS-derived envelope from an infected patient, HIV-1 4013env. To determine if ART controls viral replication over time in myeloid cells, MoM were treated daily with ART consisting of FTC/TDF/RAL. Data were analyzed using GraphPad Prism software (v5.04).

Results: Flow cytometry and immunohistochemical analyses demonstrated that MoM are systemically reconstituted with human monocytes (CD33+/CD14+), macrophages (CD33+/CD14+/CD16+) and B cells (CD19+). Detailed tissue analysis demonstrated that MoM completely lack human T cells (CD3+). MoM were susceptible to infection with macrophage-tropic HIV-1 isolates as determined by the sustained presence of vRNA in plasma, but depletion of myeloid cells did not occur during infection. In addition, vRNA and vDNA were present in all tissues analyzed including the brain where HIV antigen was confirmed. Finally, ART efficiently suppressed plasma viremia below the limit of detection.

Conclusions:
1. MoM are systemically reconstituted with human myeloid cells and devoid of T cells,
2. MoM are susceptible to HIV-1 infection,
3. HIV replication in MoM is systemic and sustained over time,
4. HIV infection does not decrease the number of peripheral blood myeloid cells in MoM,
5. myeloid cells migrate into the brain of MoM where HIV infection is established,
6. ART effectively controls viral replication in MoM.
Simian immunodeficiency virus (SIV) infection of the macaque testis - an immune privileged site

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Background: The testis is predicted to be a sanctuary site for HIV persistence, so a successful vaccine must prevent testicular infection. At least in rodents, the testis is also a site of immune privilege where innate and adaptive immune responses are suppressed. Evidence for immune privilege in the primate testis, however, is inconclusive. We have used the pigtail macaque model to characterise the changes to the testicular leukocytes population induced by SIV infection and the strength of immune responses in this region.

Methods: Testes were studied from six uninfected and four SIVmac239-infected macaques. Testes were recovered by bilateral orchidectomy 10 weeks after infection. Testis tissue samples were fixed in Bouin’s, sectioned and stained with periodic-acid Schiff reagent. Interstitial cells were isolated by collagenase digestion of remaining testis tissue and phenotyped using flow cytometry. Nested RT-PCR reactions were used to amplify the SIV env gene from testis samples. T-cell responses to mitogen stimulation were measured by intracellular cytokine staining for TNF and IFN\(\gamma\).

Results: The leukocyte population of the uninfected macaque testis consisted of >45% monocytes and macrophages, 15% CD4+ and 16% CD8+ T-cells (central and effector memory, but few naïve) and smaller populations (< 10%) of dendritic, NK and NKT cells. T-cell responses to mitogens and super-antigens were highly suppressed in the testis compared to blood (p=0.028) indicating that the primate testis is immune-privileged. SIV infection of the testis did not lead to obvious changes to spermatogenesis or testicular architecture. Infection did, however, decrease the proportion of testicular CD4+ T-cells, increase CD8+ T-cells and lead to a shift away from central memory and towards effector memory T-cells. Macrophage numbers were decreased whereas granulocytes numbers increased.

Conclusions: SIV infection decreased the numbers of testicular CD4+ and central memory T-cells, as well as macrophages, indicating that they are targets for the virus. However, T-cell responses are dramatically suppressed in the primate testis. This raises important questions about whether “nonsterilising” immunity-inducing HIV vaccines will seed HIV into this immune-privileged site and lead to eventual breakthrough infections.
An assay that precisely measures the size of the latent HIV reservoir reveals that ART-naïve individuals harbour a large pool of latently infected CD4+ T cells

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**Background:** Quantifying cells harboring latent provirus is critical to evaluating strategies to eliminate them, but the low frequency of these cells makes this very challenging.

**Methods:** We developed a novel assay to precisely measure the frequency of cells expressing multiply-spliced HIV RNA upon stimulation with PMA/ionomycin (Tat/rev Induced Limiting Dilution Assay, or TILDA). TILDA can be completed in 2 days and requires less than a million cells distributed in limiting dilutions (4 dilutions x 24 replicates, ranging from 18,000 cells to 1,000 cells), without the need for RNA extraction. We measured the size of the reservoir by TILDA in samples from 27 well-characterized subjects on ART (Eriksson, Plos Pathog 2013) and 10 ART-naïve individuals.

**Results:** During ART, we found the median frequency of latently infected CD4 T cells as estimated by TILDA to be 24 cells/million (range 1-101 cells/million), which is 48 times more than the frequency measured by the quantitative viral outgrowth assay (Q-VOA), and 6-27 times less than the frequencies given by PCR-based assays. The frequency of reservoir cells measured by TILDA correlated with several PCR based assays including total and integrated HIV DNA in PBMCs (p=0.05 and p=0.002, respectively) and HIV DNA in rectal CD4 T cells (p=0.002). TILDA values tended to correlate with Q-VOA, but the association did not reach statistical significance (p=0.095). The size of the latent reservoir measured by TILDA was significantly lower in subjects who initiated ART during the early phase of infection compared to those who started at a later stage (p=0.0089). In untreated disease, we observed that the frequency of latently infected CD4 T cells (TILDA) largely exceeds the frequency of productively infected cells; on average, the pool of infected cells was composed of 26% of productively-infected cells and 74% of latently -but inducible- infected cells, demonstrating for the first time that the majority of infected cells are transcriptionally silent even in the absence of ART.

**Conclusions:** Our results suggest that TILDA may be a reproducible, high-throughput, and sensitive approach to estimate the frequency of productively/latently infected cells. We also found that latency is efficiently established and maintained in untreated HIV-infection.
Innate Immune Responses and Function During HIV Infection

**PE1** HIV controllers have activated NK cells with a particular NK cell receptor profile and higher degranulation capacity

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**Background:** Natural killer (NK) cells are associated with the innate immune response and are important in the control of many viral infections including human immunodeficiency virus type 1 (HIV-1) infection. We studied the repertoire and function of NK cells in HIV-1-infected controllers (HIC) individuals, who maintain a clinically undetectable HIV viral load without treatment and we compared them to HIV infected viremic patients (VIR) and normal blood donors (ND).

**Methods:** Twenty-eight patients enrolled in the French ANRS CO18 HIV Controllers (HIC) cohort and infected by HIV-1 for >10 years who had never received antiretroviral treatment and in whom >90% of plasma HIV RNA load tests gave values < 400 copies per milliliter were studied. All phenotypic studies were done on fresh whole blood using a LSR II cytometer. CD107a expression on NK cells and the ICS-based assay were done on PBMC isolated from fresh whole blood and incubated with the target cell line K562. Associations between groups were compared using Wilcoxon rank sum test and by the Fisher exact test otherwise.

**Results:** HIC patients showed higher expression of CD158e (KIR3DL1/KIR3DS1), DX9 (KIR3DL1), and Nkp44 receptors compared to VIR patients or ND individuals suggesting a functional activation phenotype. Accordingly, degranulation capacity and production of IFN-γ was higher in HIC individuals. Unexpectedly, HIC individuals have lower expression of NKG2D receptor on NK cells than control groups. Degranulation and Nkp46 expression were positively correlated to CD8 T cell-mediated HIV viral replication suppression capacity whereas NKG2D expression was negatively correlated. The CD8 suppression assay was done in cocultures of HIV infected CD4 T and CD8 T cells.

**Conclusions:** These results pointed out the cooperation between innate and adaptive immunity in HIC individuals and suggest a role of NK cells in the maintaining of HIV CD8 T cell responses in HIC individuals.
Modulation of HIV-induced TLR activation by ammonium compounds: new therapeutic strategy to reduce chronic immune activation?

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Background: There are now multiple evidences that chronic activation of plasmacytoid dendritic cells (pDC) by HIV contribute to immunopathogenesis. Thus, targeting pDC and the Toll-like receptor pathway may open new therapeutic strategies for chronically activated HIV patients. Indeed, the pDC inhibitor Chloroquine is currently under clinical trial and the natural compound histamine was shown to have inhibitory properties on human pDC.

Methods: Human pDC were purified from healthy donors and cultivated in presence of HIV overnight. IFN-α production by pDC was quantified by ELISA and activation marker (TRAIL, CD80, CD83) expression was quantified by FACS. We tested natural (histamine, spermine, serotonin) and synthetic (clobenpropit) ammonium compounds on pDC activated by HIV and TLR-7 activators. Because these molecules are too small to be detected by antibodies, we synthetized a fluorescent ammonium compound (FC NH3+) allowing its detection and visualization into cells by 3 dimensional (3D) microscopy.

Results: Natural (histamine, serotonin, spermine) and synthetic (Chlobenpropit) molecules were all able to inhibit IFN-α production and activation marker (TRAIL, CD80, CD83) by HIV-stimulated pDC. Using a fluorescent ammonium compound FC NH3+ that we synthetized, we demonstrated that FC NH3+ enters the cells and colocalize with the HIV particles into pDC. Because the common characteristic of the molecules we tested was the amino NH3+ function, we synthesized an analogue in which the amino group was replaced by a carboxylate (FC CO2−). This modified molecule co-localized with HIV particle into pDC but did not inhibit IFN-α production or activation marker expression by HIV-stimulated pDC, highlighting the essential role of the amino function. Finally, we showed that ammonium compounds modified endosomal acidification leading to a lack of interaction of viral RNA with TLR-7, thus preserving pDC from HIV-induced over-activation.

Conclusions: In conclusion, we showed here that natural but also synthetic amino compound inhibited HIV-induced IFN-α production by pDC. Thus, we think that the study of amino compounds could represent efficient pDC modulators and open new strategies in TLR modulation for therapeutic use.
Virus-Specific Humoral Immunity

**PE3** Evolution of neutralizing antibodies in acute heterosexually acquired HIV-1C infections

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**Background:** Over thirty years after the discovery of HIV, there is still no potent and efficacious vaccine against the infection. The recent discovery of potent and broad neutralizing antibodies against HIV-1 has revived the interest in the search for more of these antibodies and to investigate their possible role in preventing the infection.

**Methods:** A cohort of eight HIV-1C acutely infected individuals were enrolled and followed for a period of up to 24 months. Plasma samples were collected at different time points during the follow-up and stored at -80°C. Viral RNA was extracted from the plasma of these individuals collected at or around baseline as well as 6 months post enrolment and the envelope protein, glycoprotein 160, amplified. The HIV-1C envelope gene from these individuals was cloned into pcDNA3.1D/V5-His expression vector. The envelope clones were subsequently used to co-transfect 293 T cells along with a backbone vectors to produce pseudoviruses. A standardized neutralization assay based on TZM-bl cells was used to determine the autologous neutralizing capacity.

**Results:** All but one individual’s envelope pseudotyped viruses were neutralized by some of the autologous plasma albeit very late. Viruses generated during the acute infection period displayed varying neutralization sensitivities towards autologous plasma. Potency of the neutralization increased with time post infection time point. Neutralizing antibody development lags behind as evidenced by the lack of neutralizing capacity of the plasma to the contemporaneous HIV viral envelope generated from the primary infection. While the mutated viral species present at 6 months show a similar trend but there is fast development of neutralizing antibodies that become effective 3 months later. One of the individuals showed sharp decrease of the viral load at the same time there was marked increase in potent neutralizing antibodies.

**Conclusions:** HIV-1 subtype C neutralizing antibodies from primary infection develops much later, around 5 months with significant potency increasing at around 2 years post infection. While any subsequent viral strains generated from mutation are rapidly recognized by the developing immune response, especially post introduction of antiretroviral treatment. This has given interesting pointers to HIV vaccine development and design for prevention working together with treatment.
Antiretroviral therapy preserves polyfunctional HIV-1-specific CD8 T cells with stem cell-like properties

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Background: CD8 T stem cell memory T cells (T_{SCM} cells) have been recently identified in humans, mice and non-human primates, and seem to represent the most immature memory CD8 T cell population with potent abilities to proliferate and repopulate the memory T cell pool. The presence and the function of CD8 T_{SCM} cells in HIV-1-positive individuals is unknown.

Methods: CD8 T memory stem cells were analyzed in 43 HIV-1 patients (11 with HAART-treated HIV-1 infection, 18 with untreated progressive HIV-1 infection, and 14 untreated controllers with spontaneous control of HIV-1 viremia to < 1000 copies/ml). Phenotype and function of CD8 T_{SCM} cells were assessed by flow cytometry; virus-specific CD8 T cell populations (n=32 HIV-1-specific responses, n=15 CMV/EBV/Flu-specific responses) were identified by MHC class I multimer staining or intracellular cytokine staining. 12 HIV-1 negative study subjects with n=11 CMV/EBV/Flu-specific CD8 T cell responses were analyzed as controls.

Results: Levels of T_{SCM} in total CD8 T cells were significantly decreased in untreated HIV-1 patients (p< 0.04), but not different between HAART-treated HIV-1 patients and negative control patients. The frequency of CMV-, EBV- and Flu-specific CD8 T_{SCM} cells in HIV-1-infected patients was similar to uninfected individuals, but significantly higher than the proportion of HIV-specific CD8 T cells in HIV-1 patients (p=0.009). Among all HIV-1 patients, HIV-1-specific CD8 T_{SCM} cells were most frequent in HAART-treated patients (p< 0.02). Moreover, HIV-1-specific CD8 T_{SCM} cells from these patients showed the highest degree of polyfunctionality in comparison to the other groups of patients (p< 0.03).

Conclusions: Polyfunctional HIV-1-specific CD8 T_{SCM} cells are able to persist long-term and show a relative accumulation during antiretroviral therapy when viral antigen is pharmacologically suppressed. Due to their antigen-independent persistence in HAART-treated patients, these cells may be particularly effective in targeting the reservoir of HIV-1-infected cells after pharmacological interventions that reverse viral latency.
Immunity to HIV GAG peptides and routine vaccine antigens in HIV-exposed uninfected infants in the ART era

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Background: Successful implementation of policies to prevent mother-to-child-transmission of HIV-1 infection, children born to HIV-1-infected mothers are less likely to acquire HIV-1 infection. Nevertheless, HIV-1-exposed uninfected (HEU) children have substantially increased morbidity and mortality compared with unexposed uninfected children (UU), predominantly from infectious causes. As HEU children continue to increase worldwide, we investigated the consequence of HIV exposure on the neonatal immune system and vaccine specific responses in early childhood.

Methods: 54 UU and 54 HEU infant-mother pairs were recruited at a vaccination clinic in Blantyre, Malawi. We measured GAG peptide-specific and vaccine antigen-specific (tetanus toxoid (TT), Hepatitis B and PPD) CD4+ T-cell immune responses in PBMCs by overnight IFNγ ELISPOT assay, and B-cell memory responses to diphtheria (DT) and TT by cultured ELISPOT. Baseline phenotyping on both T- and B-cells for activation (HLA-DR), senescence (PD-1) and exhaustion (CD57) markers were used.

Results: HIV-EU infants responded more frequently [HEU 15/25 Vs. UU 2/24] and to a higher magnitude [HIV-EU 2.7[0.001-41.0] Vs. UU 0.001[0.001-0.001; p=0.0001)] than UU infants to the GAG peptide pool. There were no significant differences between HEU and UU infant antigen specific T-cell responses to PHA (HIV-EU 170[34.7-453] Vs. UE 154[62-320.7]), Tetanus Toxoid (10.7[0.5-33] Vs. 8[0-20.7]), Hepatitis B surface antigen (4.6[0-39.33] Vs. 8.6[0-19.50]) and PPD (93[5.97-270.7 Vs. 38[6.417-99.83]) (all p>0.05). B-cell memory responses to DT and TTs toxoid were acquired equally in HEU and UU infants from 6 weeks pre vaccination up to 21 weeks of age after the final vaccine. Phenotyping showed HEU at 6weeks, CD4+ T-cells had higher double expression of (PD-1/CD57) (P=0.0118) which persisted at 14 weeks (P=0.0307) and fewer transitional (CD19+/CD10+) (P=0.0471) and tissue like memory B-cells (CD21-/CD27-) (P=0.0006) when compared to UU infants.

Conclusions: HEU infants frequently have immunological evidence of HIV exposure in early life which persists from 6 weeks up to 21 weeks of age. Nonetheless, these HEU infants are capable of making vaccine antigen-specific T- and B-cell memory responses of a similar magnitude as unexposed infants. Lymphocyte perturbations in both T- and B-cell populations may represent an immunological footprint of HIV exposure, with functional consequences in the long term.
Correlates of Immune Protection

PE6 A longitudinal assessment of host immunosuppressive responses in primary and chronic HIV infection

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Background: In chronic HIV infection, we previously showed an expansion of regulatory T-cells (Tregs) and enhanced immune suppression mediated by Tryptophan (Trp) catabolism into Kynurenine (Kyn) via IDO enzyme expressed in dendritic cells (DC). Here, we longitudinally assessed changes of Trp catabolism and inflammation following primary HIV Infection (PHI).

Methods: Plasma and PBMCs were longitudinally collected from PHI patients (n=41) in whom 24 remained untreated during their chronic phase (ART-naive) and 17 were ART-treated one year later. We also assessed samples from elite controllers (EC, n=12) as well as healthy subjects (HS, n=12). IDO activity marker (Kyn/Trp ratio), inflammatory cytokines, T-cell activation markers HLA-DR/CD38 and frequency of Treg, myeloid-DC (mDC) and plasmacytoid-DC (pDC) were evaluated.

Results: PHI patients had a higher Kyn/Trp ratio compared to HS and EC which further increased in the chronic phase, but normalized following ART. Accordingly, Treg frequency was increased over time for those untreated. The lower frequency of mDC and pDC in PHI and ART-naive patients was associated with higher Kyn/Trp ratio. The highest HLA-DR+CD38+ activated CD8 T-cell levels were observed during PHI followed by a decrease in chronic phase, which became comparable to EC and HS in ART-treated. Importantly, Kyn/Trp ratio was correlated with the level of CD8 T-cell activation during PHI and for those who remained untreated. In addition, Kyn/Trp ratio was positively associated with HIV disease progression markers IL-6 and IP-10 and other inflammatory cytokines IL-18 and TNF-α.

Conclusions: The progressive increased Kyn/Trp ratio observed in the chronic phase of HIV infection in contrast to a decrease in both viral load and T-cell activation, supports further contribution of tissue damage and/or myeloid-lymphoid inflammatory cross-talk, in addition to viral replication, which contribute to immunosuppression.
Mechanisms of Activation / Inflammation and Impact on Pathogenesis

**PE7 Social stress prior to SIV or SHIV infection associates with higher viral load and lower CD4 counts**

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**Background:** The tempo of disease progression in HIV-infected humans and SIV-infected macaques is quite variable among individuals, with high levels of virus replication and immune activation predicting shorter survival. Prolonged exposure to stress alters immune system function and shortens survival time in SIV-infected macaques. Macaques establish a relatively stable matriarchal hierarchy in which subordinate animals consistently demonstrate elevated markers of chronic stress compared to dominant ones. In the current study we tested the hypothesis that stress history, as dictated by the subject’s social rank prior to study assignment, predicts plasma viral load (PVL) and disease progression during chronic SIV infection.

**Methods:** All macaques originated from social groups housed at Yerkes National Primate Research Center with established rank systems. Retrospective individual animal meta-analysis was conducted on PVL and CD4 data across the acute and chronic phases of infection and the relative data were retrospectively compiled and analyzed. PVLs from five previous studies were measured by RTPCR as copies of SIV RNA/ml of plasma. From blood absolute CD4+ T-cell counts and Ki-67 expression were measured by flow cytometry.

**Results:** Sixty-two infected macaques were stratified based on the pre-infection social rank and evaluated longitudinally. In the acute phase of infection, neither virus replication nor absolute CD4+ T-cell count were predicted by social rank prior to study assignment (p>0.05). However, in chronic phases of infection, social rank prior to study assignment influenced the level of virus replication and absolute CD4 count (p<0.05). In the chronic phase of infection plasma PVL from subordinate subjects was elevated compared to that from dominant/mid ranks (mean 5.806 log10 vs. 4.213 log10 copies/ml), and absolute CD4 counts were significantly decreased in subordinate subjects compared to dominant/mid subjects (mean 348 vs. 513 cells/ul blood). Finally, CD4 Ki-67 expression increased from the acute to chronic phase more in subordinate animals compared to dominant/mid animals.

**Conclusions:** These data demonstrate that social history prior to SIV/SHIV infection influences PVL and CD4 count in chronic disease, with increased exposure to stress being associated with higher levels of virus replication, increased depletion of CD4+ T-cells, and greater increases in activation of CD4+ T-cells.
Increased immune activation and hypercoagulation after gut permeabilization in SIV-infected African green monkeys

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Background: Microbial translocation (MT) has been proposed as a major cause of increased immune activation/inflammation (IA/INFL) in HIV/SIV infection and subsequent cardiovascular comorbidities, including hypercoagulability. Our hypothesis was that alcohol administration to non-progressive African green monkeys (AGMs), which maintain a healthy intestinal barrier despite high levels of viral replication during chronic SIV infection, will result in increased gut permeability and consequent levels of IA/INFL and hypercoagulability.

Methods: Six AGMs were administered ethanol by indwelling gastric catheters for 3 months, and then intravenously infected with SIVsab92018. Alcohol administration continued for up to 6 months postinfection. We compared the levels of viral replication, T cell changes, immune activation (HLA-DR CD38 and Ki-67), MT (LPS, sCD14), coagulation (D-Dimer) and plasma cytokines in alcohol-treated infected AGMs and infected AGM controls. Microbial products and fibrosis were assessed in the liver by immunohistochemistry.

Results: Alcohol administration to AGMs altered the mucosal barrier and resulted in increased MT, as shown by the significantly higher levels of LPS and sCD14 in alcohol receiving AGMs, and increased levels of microbial products in the liver. Alcohol induced increased levels of T cell activation and proliferation and higher levels of proinflammatory cytokines (IL-1β, IL-6, IL-17) in the treated animals compared to controls. A slight increase in plasma viral loads and a delayed CD4⁺ T cell restoration in the gut and periphery were observed in the SIVagm-infected AGMs receiving alcohol. Numerous inflammatory infiltrates and increased fibrosis were observed in the liver in the alcohol-treated AGMs. Finally, induction of MT in alcohol-treated AGMs infected with SIVagm resulted in significantly higher levels of the coagulation biomarker D-dimer compared to controls.

Conclusions: Our results showed that alcohol plays a role in permeabilizing the gut and is increasing MT in AGMs. Alcohol treatment of SIVagm-infected AGMs resulted in increased systemic immune activation and inflammation, delayed immune restoration, and activation of coagulation. Our results bring direct proof that MT is one of the important mechanisms of excessive immune activation/inflammation and a major cause of cardiovascular comorbidites in HIV infected patients.
Comparison between pathogenic and nonpathogenic SIV infections and focus on mucosal tissue compartment reveal a critical role for the adenosine pathway in the control of SIV-related immune activation and inflammation

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**Background:** Adenosine (ADO) is associated with immunosuppression in multiple diseases, yet its role in modulating immune activation/inflammation (IA/INFL) in HIV-1/SIV infection is unclear. To distinguish its role in the pathogenesis of AIDS, we compared and contrasted ADO-associated markers between African green monkeys (AGMs) that control SIV-related IA/INFL and do not progress to AIDS, and pigtailed macaques (PTMs) that have aberrant IA/INFL and experience disease progression.

**Methods:** CD39/CD73 and CD26 (which promote ADO production or its breakdown to inosine) were assessed by flow cytometry on T cells from blood, lymph nodes (LNs) and intestine of AGMs and PTMs prior, during acute, early and late chronic SIVsab infection. ADO markers were correlated with T cell immune activation (CD38/HLA-DR and Ki-67), monocyte activation (sCD14 and sCD163), and inflammation (CRP and inflammatory cytokines). Mass spectrometry was used to directly assess ADO levels in LNs and intestine.

**Results:** Baseline CD39/CD73 expression on Tregs from the LNs and intestine were intrinsically elevated in AGMs and remained high throughout SIV infection. CD26 and inosine levels did not change in AGMs. In the gut, baseline ADO levels were intrinsically high in AGMs and further increased early after infection. Increased ADO levels were associated with control of IA/INFL in AGMs. Conversely, in PTMs, CD39 expression on circulating conventional T cells and Tregs significantly increased only during the late SIV infection. CD39/CD73 expression significantly increased on conventional T cells and Tregs from LNs, while the gut biopsies showed that only CD73 significantly increased on Tregs from PTMs after infection. CD26 dramatically increased in the LNs and intestine throughout progressive infection. Consequently, inosine was significantly higher in the intestine in PTMs during chronic infection and correlates with lack of control of IA/INFL in PTMs.

**Conclusions:** Our results bring the direct proof that ADO is involved in the control of IA/INFL in nonprogressive SIV infections. ADO production appears to be counteracted by a massive increase of CD26 which occurs early in progressive infections. Changes of ADO levels predominately occur in the gut during SIV infection, suggesting that future studies of the ADO pathway should focus on mucosal sites rather than in blood or LNs.
**PE10 SIV-infected rhesus macaques receiving suppressive cART have continued GI damage, inflammation, immune activation and persistent low-level viral replication within lymphoid tissues**

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**Background:** A detailed understanding of the mechanisms for long-lived viral reservoir maintenance will likely be key to designing successful strategies for an HIV functional cure. Persistent inflammation and immune activation may lead to ongoing low levels of viral replication in the setting of suppressive cART resulting in continued reservoir persistence. Major gaps still exist in our understanding of viral reservoir maintenance that are particularly difficult, if not impossible, to study in humans, however, studies in nonhuman primates provide ideal models to address this important question.

**Methods:** 5 rhesus macaques (RM) were infected with SIVmac251 and at 8 wpi received a five-drug regimen of cART (FTC/PMPA/Raltegravir/Darunavir/Ritonavir) for 20 weeks at which time the animals were necropsied with a comprehensive tissue collection for each animal. In situ hybridization, immunohistochemistry and quantitative image analysis was performed on lymph nodes (axillary, inguinal, mesenteric), spleen, GI (jejunum and colon), liver, and lung to comprehensively determine the level of persistent viral replication, GI damage (PMN infiltration; MPO staining), inflammation (MxA, TNF-α), and immune activation (Ki67) in the setting of suppressive cART.

**Results:** While immune activation (Ki67) and inflammation (MxA and TNF-α) in lymph nodes and the GI were attenuated after suppressive cART compared to pre-ART, they still remained significantly elevated compared to SIV- controls. Persistent GI damage, as measured by PMN infiltration (MPO staining), was still evident after 20 weeks of cART and significantly greater than SIV- RMs. Importantly, while the level of SIV vRNA+ cells were significantly reduced after cART evidence for persistent low-level viral replication were detected within lymphoid tissues in most animals.

**Conclusions:** Persistent GI damage, inflammation and immune activation is still evident in RMs after 20 weeks of cART and may explain, in part, why continued low-level viral replication still occurs in lymphoid tissues.
HIV Replication and Pathogenesis in Other Organs and Tissues

**PE11** Envelope glycoprotein determinants of macrophage tropism during progressive HIV-1 subtype C infection

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**Background:** HIV-1 subtype C (C-HIV) is responsible for most HIV-1 infections worldwide, but the determinants of C-HIV pathogenicity are poorly understood. In HIV-1 subtype B (B-HIV), pathogenesis has been associated with changes in the Env entry protein that allows the virus to adopt altered configurations to overcome entry restrictions imposed by low levels of CD4 on macrophages. Our objective was to elucidate the extent and determinants of macrophage (M)-tropism during progressive C-HIV infection. To do this, we used a panel of plasma-derived CCR5-using (R5) Envs (n=199) that we cloned from 20 antiretroviral therapy (ART)-naïve subjects experiencing progressive C-HIV infection over a 3-year period [Jakobsen et al. 2013, e65950].

**Methods:** 199 R5 Envs, cloned from a cohort of 20 ART-naïve subjects from Zimbabwe who progressed from chronic to advanced stages of C-HIV infection over a 3-year period, were screened for their ability to enter monocyte-derived macrophages (MDM) after being pseudotyped onto luciferase-expressing reporter viruses. CD4 and CCR5 dependencies of genetically-related M-tropic and non-M-tropic C-HIV Envs were determined using the 293-Affinofile affinity profiling system. Viruses were also tested for sensitivity to the mAb b12 and the CCR5 antagonist maraviroc (MVC).

**Results:** 7% of C-HIV Envs (14/199 Envs from 10/20 subjects) could efficiently enter MDMs, and their emergence in plasma was not related to the stage of infection. Of these, 50% (n=7) displayed enhanced ability to interact with low CD4 levels and increased sensitivity to neutralization by b12. However, 50% of M-tropic C-HIV Envs (n=7) could not use low CD4 levels and did not have increased sensitivity to b12. M-tropism among the latter group was associated with Env changes in the coreceptor binding domains, but inhibition studies with MVC showed that they do not interact with CCR5 differently than non-M-tropic Envs.

**Conclusions:** Our results show that M-tropic Envs rarely circulate during progressive C-HIV infection. When M-tropic viral variants do emerge in plasma, their Env phenotypes may be characterized as (1) those having reduced CD4-dependence similar to most M-tropic B-HIV Envs, or (2) those able to efficiently enter MDM via an alternate mechanism. This alternate mechanism (or mechanisms) may be unique to C-HIV strains, and warrants further investigation.
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Background: While effective therapy allows most to live long, productive lives, HIV remains incurable due to virus dormant in long-lived cells, established within days of infection and capable of reigniting infection. One method of eliminating latent virus is to awaken dormant HIV, then target the infected cells: the kick and kill strategy. The CNS compartment is an important reservoir in HIV, yet sensitive to the neurotoxic effects of both HIV and antiretroviral treatment. The class I histone deacetylase inhibitor (HDACi), vorinostat (VOR) disrupts latency and upregulates HIV RNA expression, which could be a first step towards practical HIV eradication therapies. However the effect of this intervention within the CNS is unknown, and de novo virion expression of viral protein production could have an adverse effect on neurocognitive function. Here we present the first data on neurocognitive functioning in patients receiving VOR during a clinical trial, demonstrating no change in function with repeated administration.

Methods: Five consenting patients received VOR 400 mg daily M-W for 4 weekly cycles, followed after a 5-6 week rest period by another 4 weekly cycles. Neurocognitive performance was assessed at baseline and study end in the domains of Language (WRAT-4 Reading), Motor (Grooved Pegboard), Learning (HVLT-R), Memory (HVLT-R delayed recall), Speed of Processing (Stroop, Trailmaking A, Digit Symbol), Attention/Working Memory (Symbol Search), and Executive functioning (Stroop interference, Verbal fluency, Trailmaking B).

Results: VOR was well tolerated, and HIV RNA induction (>1.5-fold) was only seen in three patients in 4 of 6 assays. No significant change in average total z score was found (black dotted line, Fig. 1), the mean baseline total z was -.29 (SD=.21) and the follow-up score showed expected slight improvement at -.12 (SD=.45).

Conclusions: In VOR use for HIV eradication of latent virus with over 22 doses across 4 months, no significant change in neurocognitive performance was found, indicating initial safety for the CNS. However, using this dosing schema, repeated and potent induction of viral expression was not seen, limiting the relevance of this neurocognitive evaluation. Neurocognitive assessments may assist in the evaluation of host effects as improved strategies to disrupt latent HIV infection evolve.
Naïve CD4+ T-cells are resistant to HIV infection in vitro in the presence of homeostatic signals

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Background: HIV persists in both long-lived memory and naïve CD4+ T-cells during suppressive antiretroviral therapy. Naïve T-cells are relatively resistant to HIV infection in vitro and it remains unclear how this reservoir is established and maintained. We hypothesised that naïve T-cells would become susceptible to infection following stimulation with interleukin-7 (IL-7) or dendritic cell (DC) co-culture as both are stimuli for homeostatic proliferation.

Methods: Total, CD31+ or CD31- naïve CD4+ T-cells were isolated using FACS sorting and cultured with IL-7 (50ng/ml) alone, or with immobilised anti-CD3 (5µg/ml) or blood DC (1:10) and infected with both laboratory and primary isolates of HIV-1 or HIV-2. HIV-DNA was quantified using real-time PCR. Markers of T-cell activation (CD69, HLA-DR), cell cycle entry (Ki67), proliferation (CFSE) and IL-7 responses (STAT5, Bcl-2) were measured using flow cytometry. Efluor670 labelled naïve T-cells were co-cultured with plasmacytoid (p) or myeloid (m) DC and infected with (NL(AD8)ΔnefEGFP). Productive infection was quantified by EGFP expression. SAMHD1 expression was measured using real-time PCR and western blot.

Results: Following HIV infection with all isolates, HIV DNA was detected in both CD31+ and CD31- naïve T-cell that were cultured in IL-7 and anti-CD3 but not when cultured with IL-7 alone (n=3). Addition of IL-7 alone to naïve T-cells led to clear increases in STAT5 phosphorylation (n=3) and Bcl-2 expression (n=4) compared to media alone, but no increase in Ki67 or proliferation (n=6). When cultured with IL-7 and anti-CD3, CD69, HLA-DR and Ki67 expression increased however these changes were associated with an increase in CD45RO expression consistent with conversion to a memory phenotype (n=3). Following infection of naïve T-cells co-cultured with mDC, EGFP+ T-cells were detected (median=5.58% (IQR=1.45-6.22), n=5), but again this was associated with a change to a memory phenotype. The host restriction factor SAMHD1 was detected in both activated and resting memory and naïve CD4+ T-cells. HIV-2, which contains Vpx known to degrade SAMHD1, was unable to infect naïve T-cells in vitro.

Conclusions: Homeostatic signals like IL-7 and DC were unable to alleviate blocks to naïve T-cell infection in vitro, suggesting alternative pathways are required to establish this stable long-lived reservoir.
Viral Mechanisms of Persistence and Latency

**PE14 Tropism alterations of HIV-1 subtype C strains for CD4+ T cell subsets**

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**Background:** Despite the majority of individuals with HIV being infected with subtype C (C-HIV) and residing in resource-constrained nations, the bulk of research has been conducted on subtype B. The pathogenesis of C-HIV is poorly understood, and it is largely unknown whether changes in viral tropism, and potentially the size of the viral reservoir, occur. The objective of this study was to examine whether changes in the viral envelope (Env) during disease progression alter the cellular tropism of the virus, potentially affecting the development of viral reservoirs.

**Methods:** This study utilized two participants from a unique ART-naïve Zimbabwean cohort, who progressed from chronic to advanced stages of disease over approximately 3 years (Jakobsen et al 2013). Envs were cloned from longitudinal plasma samples and Env-pseudotyped GFP reporter viruses were used to infect CD4+ T cells from 5 healthy donors. We developed and validated a novel T cell assay for the detection of the newly described stem memory T cell subset (TSCM). Infection of T cell subsets (naïve, EMRA, TSCM, CM, TM, EM) were determined by flow cytometry.

**Results:** Both participants maintained CCR5 using viruses, which were poorly macrophage tropic, throughout the study. Despite a substantial decrease in CD4+ T cell counts, there was minimal change in the infection of T cell subsets during chronic disease progression (3 years). Although not significant, there was a trend for an increase in EM and decrease in naïve T cells to be susceptible to infection as disease progressed. The infection of HIV viral reservoirs including CM, TM and TSCM cells was maintained by chronic viruses and there was a higher than expected infectivity of TSCM cells (6% - 4% total CD4+ T cells infected).

**Conclusions:** The susceptibility of TSCM cells to HIV-1 infection, their contribution to the viral reservoir and long-lifespan highlights the importance of targeting these cells in future therapeutic strategies.
HIV-1 establishes transcriptional latency in astrocytes in vitro and is responsive to HDACi

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\textbf{Background:} HIV-1 penetrates the central nervous system (CNS) during early infection, establishing a persistent infection. While macrophages and microglia represent the sites of productive HIV-1 infection, astrocytes undergo a restricted/latent infection. We recently demonstrated that astrocytes are extensively infected in vivo and may therefore constitute a significant potential reservoir of HIV-1 within the CNS. Here, we generated an \textit{in vitro} model of latency within an astrocyte cell line and determined the response of HIV-1 to histone deacetylase inhibitors (HDACi).

\textbf{Methods:} A lentiviral packaging system was used to generate vesicular stomatitis virus (VSV)\textsubscript{g} Env-pseudotyped viral particles that contained a HIV-1 integrating provirus (devoid of Env and encoding a luciferase reporter) with an interchangeable LTR. The astrocyte cell line, SVG, were infected with the pseudotyped virus containing the NL4.3 LTR in the presence or absence of the integrase inhibitor raltegravir, and luciferase activity was measured every 24 hours for 7 days. Additionally, cells were treated with various activators (PHA/PMA, HIV-1 Tat) and the HDACi (trichostatin A, vorinostat, romidepsin, entinostat, panobinostat) and activation from latency was assessed by measuring luciferase activity.

\textbf{Results:} SVG cells supported entry and integration of the reporter provirus and raltegravir blocked >95\% of the signal, confirming the majority of the luciferase signal was derived from integrated provirus. Following infection, there was a gradual increase in luciferase activity until day 3, followed by a sharp decline to basal levels by day 5 onwards. Luciferase activity significantly increased following the addition of all compounds, including all HDACi (ranging from 50 to 500-fold increase), with panobinostat and trichostatin A inducing the highest expression of luciferase (mean 425- and 511-fold increase, respectively).

\textbf{Conclusions:} This \textit{in vitro} model can mimic latent infection in an astrocyte cell line and is a useful tool to assess the activity of primary CNS isolates and their response to transcriptional activators. The clear activity of HDACi in this \textit{in vitro} astrocyte model may have implications for the use of these compounds in current clinical trials. The potential to activate the CNS HIV-1 reservoir needs to be considered when designing HIV-1 cure and eradication strategies.
**PE16** Alternative RNA splicing in latently infected T cells generates chimeric cellular:HIV mRNAs with the potential to generate Tat and reactivate infection

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**Background:** Post-integration latent infection of resting CD4 T cells is a major barrier to HIV cure in HIV-infected patients on antiretroviral therapy. Post integration latency is maintained by transcriptional interference, where a strong upstream cellular promoter overrides transcription from the HIV LTR. However, since HIV predominantly integrates into the introns of transcriptionally active genes, alternative splicing of read-through cellular transcripts containing the HIV pre-mRNA could lead to chimeric cellular:HIV mRNAs. The cellular: tat mRNAs, if translated, would activate transcription given Tat is a potent transcriptional activator.

**Methods:** Primary resting CD4 T cells were incubated with the chemokine CCL19 and infected with NL4.3 and latency was established after 4 days. RNA was extracted from latently infected primary T-cells and the latently infected T cell lines, J-Lat 6.3 and ACH2. Extracted RNA was amplified by RT-PCR using primers for Alu repeats (primary cells) or cellular exons (cell lines) and tat exon 2 (reverse primer) that contains the transcriptional activator function. The products were analyzed by cloning, sequencing and deep sequencing. A model chimeric gene was generated to show that Tat can be translated from cellular: tat mRNAs by an internal ribosome entry site (IRES)-like mechanism.

**Results:** Chimeric cellular: tat mRNAs were readily detected in both latently infected primary T-cells and the latently infected cell lines. Sequencing of chimeric mRNAs from the T cell lines revealed the expected splicing at cellular exons, but also at intronic cryptic sites adjacent to the proviral integration site, followed by splicing at HIV-1 splice donor (SD)₁/splice acceptor (SA)₃ to generate Tat coding sequence. In addition, Tat protein could be expressed from similar chimeric mRNAs by an IRES-like mechanism.

**Conclusions:** During read-through cellular transcription in latently infected T cells, HIV provirus RNAs encoding tat are generated by alternative splicing. An IRES-like element in tat allows translation of this mRNA and expression of functional Tat protein. Since Tat is central to both the establishment and maintenance of latent infection in resting CD4 T cells, factors which affect either transcription, splicing or translation of Tat from chimeric RNAs could be targeted to develop novel strategies for activation and clearance of latently infected cells.
Activation of antigen-specific CD4+ T cells impacts on the establishment of the HIV DNA reservoir

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Background: Memory CD4+ T cells containing integrated HIV DNA are a major barrier to clearance of HIV from individuals receiving ART. We quantified and sequenced HIV DNA in memory CD4+ T cells sub-divided by their antigenic specificity, hypothesizing the varying exposure patterns of CD4+ T cells to CMV, HIV and tetanus toxoid (TT) antigens would result in differences to the HIV DNA these populations contain.

Methods: Antigen-specific memory CD4+ T cells (n=3 donors) were purified by cell sorting after being identified by induced up-regulation of co-expression of CD25 and CD134 following a 44-48 hour incubation of PBMC or WB with individual antigens: HIV gag peptide pool; CMV lysate; or TT. Control populations including: lymphocytes; memory CD4+ T cells; naïve CD4+ T cells; and B cells were collected. Total HIV DNA was measured by real time PCR. Amplified HIV gag PCR products from CD4+ T cell subsets, controls, and HIV RNA from longitudinal plasma samples, were cloned and sequenced. Evolutionary divergence was measured using the Maximum Composite Likelihood model with gamma distribution rate variation modelling.

Results: The frequency of antigen-specific CD4+ T cells were: CMV (0.7-7.1% of total CD4s), HIV (0-0.62%), and TT (0.13-0.68%), with cell yields ranging from 0-148,000. HIV DNA was detected in all antigen-specific CD4+ T cell subsets and controls except B cells: lymphocytes (median = 47 copies/10⁶ CD4s); memory (203) and naïve (32) CD4+ T cells; CMV- (174), TT- (130) and HIV- (60) specific CD4+ T cell subsets. No significant differences were identified and one patient’s real time PCR signals were below the dynamic range of the assay. Mean nucleotide distances between clonal sequences from individual antigen-specific CD4+ T cell subsets were 0.020, 0.007, 0.002 substitutions per site for CMV, TT and HIV respectively.

Conclusions: The low yields and HIV DNA levels within isolated CD4+ T cell subsets highlight the difficulties faced by this approach. The trend to higher levels of HIV DNA and genetic diversity in CMV- relative to TT- and HIV-specific CD4+ T cells suggests that the chronic presence of CMV antigen and activation of responding CD4+ T cells increases their susceptibility to repeat infection by HIV.
**Background:** HIV/AIDS is treatable but not curable because of a persistent reservoir in resting CD4 T cells that is maintained during antiretroviral therapy (ART). Reservoir CD4 cells produce low-level viral protein Gag during ART but is insufficient to support virus spread and evolution. Gag induced autophagy that generally inhibits apoptosis. We proposed that Gag-mediated autophagy might protect reservoir CD4 cells from cell death and immune clearance, which might be an important mechanism for HIV persistence.

**Methods:** Several single-cycle HIV reporter viruses carrying GFP (pNL4.3-Δenv-GFP, pNL4.3-Δenv-Δnef-GFP and pNL4.3-Δgag-pol-Δnef-GFP) were prepared by 293T cell transfections with a complementing plasmid supplying Env or Gag-Pol in trans. Resting primary CD4 T cells were separated by negative selection and directly infected with several single round HIV reporter viruses by spinoculation. Spinoculated resting CD4 T cells were cultured for two days and treated without or with apoptosis inducer Etoposide for 24 hours, then reactivated with Protein Kinase C activator (prostratin) for 24 hours. Frequency of GFP+ cells was detected by flow cytometry. Plasmid expressing a Rev-independent Gag was transfected into resting CD4 T cells by nucleofection method. Cell apoptosis was examined by AnnexinV staining. Protein expression was detected by western blot.

**Results:** Using a HIV latency model, directly infected, resting CD4 T cells produced low levels of Gag without GFP expression after two days infection. Prostratin treatment activated latent proviruses and increased GFP expression. Cells infected with pNL4.3-Δenv-GFP and pNL4.3-Δenv-Δnef-GFP, but not pNL4.3-Δgag-pol-Δnef-GFP (or a control lentiviral vector expressing only GFP), are resistant to apoptosis with increased GFP+ cell frequency after Etoposide treatment. The autophagy inhibitor Chloroquine reduced apoptosis resistance in these latently infected cells. Transient Gag expression induced autophagy in resting CD4 T cells. Primary CD4 T cells transfected with a plasmid expressing Gag-GFP fusion protein are more resistant to Etoposide -induced apoptosis than untransfected cells. The apoptosis resistance was blocked by autophagy inhibitor Chloroquine.

**Conclusions:** Our finding that Gag-mediated autophagy promoted survival of HIV latently infected resting CD4 T cells provide novel mechanistic insights into HIV persistence and might lead to new therapeutic strategies for eradicating latently infected cells.
Host Cellular Factors and Latency

**PE19 Drug leads that inhibit Vif and enable APOBEC3G are broadly neutralizing of HIV1 clades and drug-resistant strains**

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**Background:** HIV Viral infectivity factor (Vif) evokes the destruction of the host restriction factor known as APOBEC3G (A3G). Vif dimerization has been shown to be essential for Vif binding to A3G and A3G degradation. Experimental data show that in the absence of Vif, virion-assembled A3G will hypermutate proviral DNA during reverse transcription. The open questions are: can Vif be successfully drugged and by sparing A3G, will hypermutation activity be sufficient to inhibit viral replication?

**Methods:** High throughput screening (HTS) assays were developed using FRET for Vif dimerization. The screens were used to select compounds with dose-dependent signals by preventing Vif FRET. A secondary assay for Vif-dependent A3G degradation was used to identify compounds that preserved A3G. These were triaged for the ability to inhibit pseudotyped HIV replication and to have low cytotoxicity. Ion torrent sequencing of integrated viral genomes revealed extensive hypermutation characteristic of A3G preferences. Following medicinal chemistry, a lead was tested in a seven day spreading infection in PBMC.

**Results:** The Vif dimerization FRET assay provided a robust HTS method applicable to a large library of drug-like molecules. Quantitative HTS followed by orthogonal secondary screen and cytotox counter screening enabled selection of limited number of chemistries for pseudo typed viral testing. A lead (SMVDA) was selected with nanomolar IC\(_{50}\). By impairing Vif dimerization, A3G degradation was reduced and viral particle incorporation of A3G was enhanced. Proviral DNA isolated form target cells showed numerous tracts of dG to dA hypermutation that corresponded to multiple nonsense codon and sense changes. 17 different clades and 8 drug resistant strains of HIV infecting PBMC were sterilized by seven days following a single dose of SMVDA.

**Conclusions:** Drugging Vif led to massive dG to dA hypermutation of HIV proviral DNA, such that the protein coding capacity of the virus would be severely compromised. Inhibitors of Vif are broadly neutralizing and inhibited all drug resistant strains of HIV tested. Drugging Vif therefore is achievable and the data suggest that this will serve as a firewall for viremia induced by activating reservoirs as well as a solution for rescue and salvage therapies.
Co-culture of antigen presenting cells can induce latency in resting CD4+ T-cells

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Background: Latent infection of resting CD4+ T-cells is the major barrier to HIV eradication. We have shown that latent infection in resting CD4+ T cells is facilitated by co-culture with myeloid dendritic cells (DC). The aim of this study was to determine if all antigen presenting cells (APC) share the ability to induce T-cell latency and to identify surface molecules on APC that are critical to establishing latency.

Methods: Resting CD4+ T-cells labeled with the proliferation dye eFluor670 were cultured alone or with syngeneic mDC subsets (CD1c+, CD141+, SLAN+), plasmacytoid DC (pDC) or monocyte subsets (CD14hi and CD16hi) for 24h prior to infection with a CCR5-tropic, EGFP-reporter virus. Non-proliferating (eFluor670hi), non-productively-infected (EGFP-) CD4+ T-cells were sorted on day 5 post-infection. Pre- and post-integration latency was detected by measuring EGFP expression following activation with αCD3 and αCD28 in the absence or presence of the integrase inhibitor L8, respectively. Differential gene expression between the different APC was determined using Illumina microarrays and confirmed using RNAseq.

Results: Productive infection was higher in resting CD4+ T-cells co-cultured with all APC compared to T-cells alone (p=0.03, n=6). CD1c+, SLAN+ mDC and CD14hi monocytes were all able to induce both pre and post-integration latency in resting CD4+ T-cells (p< 0.05, n=6). pDC did not induce pre or post-integration latency (p>0.1, n=6). Using gene arrays, we compared APC subsets that induced post-integration latency (CD1c+, SLAN+, CD14hi monocytes) to those that didn’t induce latency (pDC) and identified differential expression of 27 genes for surface and secreted proteins. These include pathogen recognition proteins C-type lectin 7 A (Clec 7A), signal-regulatory protein beta 1 (SIRPβ1), G protein-coupled bile acid receptor 1 (GPBAR1) and molecules involved in negative regulation, including transmembrane protein 89 (TMEM89), sialic acid binding Ig-like lectin 10 (Siglec10) and immunoglobulin-associated beta (CD79β).

Conclusions: Several subsets of mDC, as well as CD14hi monocytes, can induce latent HIV infection in resting CD4+ T-cells and may contribute to the generation of HIV latency in vivo. Future approaches to eliminate latent infection may include blocking T-cell-APC interactions that are required to establish or maintain latency.
Possible clearance of transfusion-acquired nef-deleted attenuated HIV-1 infection by a long-term non-progressor with CCR5 Delta32 heterozygous and HLA-B57/DR13 genotype

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Background: We have studied an elite HIV+ long-term non-progressor, subject C135, who was infected 33 years ago through transfusion with an attenuated nef/3’LTR-deleted HIV-1 variant, and has maintained undetectable plasma viral load and steady CD4 T cell counts, in the absence of therapy. Uniquely, subject C135 combines 4 factors separately associated with control of viremia: nef-deleted HIV-1; HLA-B57; HLA-DR13; and heterozygous CCR5 D32 genotype. Therefore we studied in detail viral burden and immunological responses in this individual.

Methods: Proviral HIV-1 DNA in PBMC and purified memory CD4+ T cells from either PBMC or from gut biopsy samples was studied by gag DNA PCR. Nef/LTR alleles were studied by nested PCR. HIV-specific antibodies were studied by Western blotting, and HIV-specific CD4+ and CD8+ T lymphocyte responses were measured by proliferation and cytokine production in vitro.

Results: PCR amplification of DNA from PBMC samples from 1996, 15 years after transfusion from an HIV+ donor showed nef alleles with gross deletions related to sequences from the blood donor, indicating established infection. However, infectious HIV-1 has never been recovered from PBMC cultures, and in samples from 1997 onwards, nef alleles could not be detected. HIV-1 gag DNA could not be detected by PCR in PBMC, by 4 different assays, nor in DNA extracted from purified memory CD4+ T cells from either PBMC or gut biopsies. Serologically, C135 has maintained a consistently weak anti-p18, -p24 and -gp120 antibody response. At the cellular level, C135 had a substantial in vitro CD4+ lymphocyte proliferative response to a previously described HLA-DR13 restricted HIV-1 p24 peptide epitope, WMNPNPPVGEIYK. This CD4 response also augmented an in vitro CD8+ lymphocyte proliferative response to a well-described immunodominant HLA-B57 restricted p24 peptide epitope TSTLQEQI-GW. Reduced CCR5 expression on CD4+ T cells was confirmed by flow cytometry.
Conclusions: Early PCR results and immunological responses suggest that infection with a poorly replicating nef-deleted HIV-1 resulted in very limited viral burden. Subsequently, over 3 decades without therapy, subject C135’s decreased CCR5 expression, and HLA-B57 restricted gag-specific CD8 immune response, supported by an HLA-DR13 restricted helper CD4 T cell proliferative response, appear to have cleared his attenuated HIV-1 infection.
**PE22 Targeting HIV splicing and translation in latently infected T cells: contributing to a cure**

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**Background:** In latently infected memory CD4+ T cells, the HIV provirus predominantly integrates into introns of transcriptionally active genes where it is subject to transcriptional interference, where read-through transcription and splicing can incorporate HIV RNA into mature cellular RNAs. HIV-1 proviruses may show stochastic fluctuations in expression depending on levels of Tat protein. Activating alternative splicing either at the HIV A3-A7 3’ss or at the cellular gene flanking the HIV provirus would lead to the incorporation of Tat-exon2, which codes for a 72aa Tat protein. Studies from our team have revealed a novel expression pathway of Tat from chimeric cellular-HIV RNAs, mediated by an Internal Ribosome Entry Site (IRES) present in Tat-exon2. To understand the function of Tat IRES, detailed knowledge of the RNA structure and the cellular factors that binds to these chimeric RNAs is required.

**Methods:** The folded structure of IRES-active tat mRNA was determined by chemical probing experiments (SHAPE). Cellular proteins binding Tat IRES were purified by fusing 3 binding sites for the MS2 coat protein to the 3’ end of tat mRNAs with and without native IRES. The RNP complexes were formed by incubation with a protein extract from a T cell line model of latent infection (J-Lat clone 6.3). The proteins were then identified by 1D gel electrophoresis followed by mass spectrometry analysis (nanoLC-MS/MS).

**Results:** 2D structure analysis of tat mRNAs by SHAPE revealed that Tat-exon2 harbours an IRES element that folds independently of the 5’UTR region. Sequence alignment of Tat-exon2 from 2233 HIV strains revealed highly conserved sequences near the Tat start-codon. We confirmed the importance of these sequences on IRES-mediated Tat translation by RNA transfection in TZM-bL cells followed by luminescence assay. Several splicing and translation factors were identified by mass spectrometry analysis: DDX3, RHA, hnRNP U, RBM39, NSRP1, LSm14A, DENR. The importance of these proteins on virus production levels and translational efficiency was assessed by p24-ELISA and confirmed by siRNA knockdown.

**Conclusions:** Our study revealed cellular proteins important in IRES-dependent and IRES-independent expression of Tat that may contribute to reactivation of latent HIV. These proteins will be assessed as drug targets.
Tissue Reservoirs

In vivo analysis of HIV persistence and immune responses in the brain

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\textbf{Background:} HIV infection is associated with neurocognitive impairment due to persistent viral infection in the brain, a major reservoir of HIV in patients undergoing antiretroviral therapy (ART). We used BLT humanized mice to study infection and the effect of ART on viral persistence and immune responses in the brain.

\textbf{Methods:} Brains were harvested from uninfected and HIV-infected animals. Cells were isolated via Percoll gradient centrifugation after perfusion for flow cytometry and viral RNA PCR analysis. Human-specific antibodies to CD45, CD3, CD4, CD8, CD19, CD33, CD14 and CD16 were used to identify immune cells. JR-CSF and CH040, two CCR5-tropic HIV-1 isolates, were used for infections. To evaluate the ability of ART to mitigate both the cellular and virological effects of HIV in the brain, mice were treated daily with ART consisting of TDF/FTC/DTG or FTC/TDF/RAL. Data were analyzed using GraphPad Prism software (v5.04).

\textbf{Results:} The brains of all BLT mice are repopulated with human T, B, and myeloid cells. Cell-associated HIV RNA was found in the brains of all infected mice (average of $1.9 \times 10^5$ RNA copies/$10^5$ cells). Co-culture of cells from infected brains with activated PBMC resulted in robust viral outgrow demonstrating the presence of replication competent virus. Also in the brain, HIV infection resulted in a significant decrease in the numbers of CD4$^+$ T cells ($p<0.001$) and the CD4:CD8 T cell ratio ($p<0.001$). These changes were evident as early as 17 days post-infection ($p=0.012$). The decrease in the CD4:CD8 ratio was due in part to an influx of CD8$^+$ T cells into the brains of HIV-infected animals. There were no differences in the numbers or phenotype (classical v. intermediate) of myeloid cells from infected or uninfected brains. ART significantly decreased the viral burden in the brain ($p=0.009$) and restored the CD4:CD8 ratio ($p=0.007$).

\textbf{Conclusions:}
1. HIV persists in the brains of BLT humanized mice,
2. HIV induces CD4$^+$ T cell depletion in the brain,
3. there is an influx of CD8$^+$ T cells in response to infection and
4. ART results in a reversal of both CD4$^+$ T cell depletion and CD8$^+$ T cell influx into the brain.
During the course of HIV infection tissue-resident effector memory CD4+ T cells are depleted but tissue-resident central memory CD4+ T cells are preserved

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Background: HIV preferentially infects and depletes memory CD4+ T cells. Tissue-resident memory T cells (T_{RM}) are a recently characterized subset of memory T cells that generate optimal immunity to site-specific pathogens. T_{RM} are characterized by expression of CD69 and are present in lymphoid and non-lymphoid tissues (Sathaliyawa-la et al Immunity 2013). Currently, studies of T_{RM} in humans are limited to biopsied and cadaveric tissue; T_{RM} do not recirculate and are virtually absent from peripheral blood. Therefore, despite their potential importance to HIV persistence, the effect of HIV infection on the T_{RM} compartment has not been evaluated. To address this critical need, we performed a detailed characterization of the human T_{RM} compartment in uninfected and HIV-infected humanized BLT mice.

Methods: We performed a flow cytometric analysis of peripheral blood and cells isolated from the tissues of uninfected and HIV-infected BLT mice. HIV-infected BLT mice were exposed to a CCR5-tropic strain of HIV. Human memory T cells (CD3+CD45RO+) that expressed CD69 were classified as tissue-resident (T_{RM}; CD69+). T_{RM} were further defined by their expression of CCR7: CCR7- (effector memory; rT_{EM}) and CCR7+ (central memory; rT_{CM}).

Results: As observed in humans, T_{RM} were present in lymphoid and non-lymphoid tissues of BLT mice but were virtually absent from peripheral blood (p< 0.0001). The tissue distribution of rT_{EM} and rT_{CM} in BLT mice was also similar to human cadaveric tissue; rT_{CM} were primarily distributed in secondary lymphoid tissues while rT_{EM} were more abundant in non-lymphoid tissues. Finally, during the course of HIV infection, CD4+ rT_{EM} were significantly depleted from all tissues examined (spleen: p=0.0043; lymph nodes: p=0.0458; bone marrow: p=0.0071; lung: p=0.0144; liver: p< 0.0001). In contrast, CD4+ rT_{CM} were not significantly reduced (spleen: p=0.1245; bone marrow: p=0.0779; lung: p=0.1107) with the exception of the lymph nodes (p=0.0109) and liver (p=0.0027) where they were modestly decreased.

Conclusions: Collectively, our results demonstrate that BLT mice possess human T_{RM} and that their distribution recapitulates the human condition. More importantly, our data reveals that CD4+ rT_{EM} are preferentially depleted during HIV infection whereas rT_{CM} are maintained. Our results indicate that rT_{CM} are important targets for destruction to achieve HIV eradication.
Preferential infection of central memory T-cells that express CCR6 and CXCR3 in HIV-infected patients on ART may contribute to a larger HIV reservoir in rectal tissue

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**Background:** We have previously shown that HIV latency can be established in vitro following direct infection of resting CD4+ T-cells in the presence of chemokines binding the chemokine receptors (CKR) CCR7, CXCR3 (X3) or CCR6 (R6). Given that these CKR are also critical for homing of CD4+ T cells to tissue, we asked whether there was a relationship between expression of these CKR, their ligands and integrated HIV DNA in blood, rectal and lymph node (LN) tissues in patients on ART.

**Methods:** HIV-infected patients on ART with HIV RNA < 40 copies/ml for ≥3 years were recruited. Leukapheresis (n=19), rectal (n=19) and/or inguinal LN biopsies (n=7) were performed. Chemokine expression was examined in plasma using Luminex and in tissue using RT-qPCR for mRNA expression of CCL19 and CCL21 (ligands for CCR7), CXCL9/10/11 (ligands for CXCR3) and CCL20 (ligand for CCR6). Chemokine receptor expression on CD4+ T cells was measured by flow cytometry. Integrated HIV DNA was measured by Alu-LTR qPCR.

**Results:** Patients had a median CD4 of 651 cells/µl (IQR 408-964). In blood, the frequency of integrated HIV DNA was highest in X3+R6+ central memory (CM) CD4+ T cells compared to X3+R6- CM, X3-R6+ CM, X3-R6- CM and naïve T-cells (p< 0.001 for all comparisons). The proportion of memory CD4+ T cells that were X3+R6+ was highest in rectal tissue (median=68%) compared to LN (12%). Rectal tissue also expressed 13 fold more CCL20 mRNA (CCR6 ligand) than LN tissue (p< 0.0001). Integrated HIV DNA was highest in rectal CD4+ T-cells compared to blood (p=0.0002) and LN CD4+ T cells, and positively correlated with rectal CXCL10 (p=0.007) and CXCL11 (p=0.019) mRNA (ligands for CXCR3). In LN, the frequency of integrated HIV DNA in LN CD4+ T cells did not correlate with LN chemokine mRNA expression nor the frequency of LN memory cells expressing X3 and/or R6.

**Conclusions:** Preferential infection of X3+R6+ CM and/or recruitment of these cells to rectal tissue via high expression of ligands to CXCR3 and CCR6 may explain the larger HIV reservoir in rectal tissue observed in patients on ART. Interventions that target CXCR3 and/or CCR6 could prove useful in curative strategies.
Measurement of Reservoirs

**PE26 Continued decay of HIV DNA species during 3-years of ART is not reflected by decay of HIV RNA in plasma**

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**Background:** The HIV DNA reservoir in CD4+ T cells is a major barrier to the eradication of HIV from infected individuals. To monitor the size and persistence of this reservoir, we measured plasma HIV RNA and CD4+ T cell associated HIV DNA species in individuals receiving three years of an antiretroviral therapy (ART) regimen containing the integrase inhibitor raltegravir.

**Methods:** The PINT study measured HIV DNA and RNA following the initiation of ART in 8 primary (PHI) and 8 chronic (CHI) HIV infected, treatment naïve individuals. The extension phase measured HIV nucleic acids at 24, 52, 78, 104, 130, and 156 weeks of ART. Single copy plasma viral load (pVL) and CD4+ T cell associated total, integrated and 2-LTR HIV DNA were quantified by real time PCR. Decay dynamics were modelled using linear mixed effects methods.

**Results:** Between weeks 24 and 156 total, integrated, and 2-LTR HIV DNA all exhibited mono-phasic decay with half-lives (with standard error) of 1,337 (SE 279), 828 (SE 119) and 772 (SE 226) days respectively. The slope of decay of each HIV DNA species was significantly different from zero, but relative differences in decay rates were not significant. Plasma HIV RNA decayed with a half-life of 1,008 (SE 580) days but was not significantly different from no decay. Total and integrated HIV DNA levels were significantly higher in CHI relative to PHI for every time-point measured (p< 0.05), however plasma HIV RNA (p< 0.05) and 2-LTR HIV DNA (p< 0.05) differences were only significant at weeks 78 and 104.

**Conclusions:** The differences in decay rates observed for CD4+ T cell associated HIV DNA species and pVL suggests a disconnect between these two sources of HIV nucleic acids. The higher levels of total and integrated HIV DNA in CHI relative to PHI were maintained for 3 years post ART initiation, but this was not reflected by differences in pVL. CD4+ T cell associated HIV DNA levels in individuals receiving ART may therefore not reflect the HIV reservoir responsible for release of HIV into the plasma.
PE27 LB Distinct transcriptional regulators associated to the control of HIV reservoirs in central-memory CD4 T cells from Elite Controllers and long-term ARV treated patients or after IL-7

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**Background:** Control of HIV reservoirs and latency is a multifactorial process depending on a tight activation/repression balance in immune cells. We showed in Elite Controllers (EC) central-memory CD4 T cells a striking over-expression of Blimp-1, a T-cell transcriptional repressor, which negatively correlates with HIV reservoirs and transcripts levels (A de Masson, AIDS 2014) while Blimp-1 was shown to repress HIV transcription. We therefore investigated whether HIV reservoirs associated with long-term ARV or immune-interventions share molecular signatures with the EC model of functional cure.

**Methods:** Resting CD4+ TN, TCM, TTM, TEM subsets were sorted from PBMCs of 7 EC (ANRS-CO15 cohort), 6 chronically-infected patients ARV-treated (median 12 years) enrolled in the Eramune-1 trial and studied before or 1 year after IL-7 infusions, and 7 un-infected donors (UI). Subsets were analysed for: 1) HIV-DNA quantification; 2) HIV-inducibility after anti-CD3+CD28+IL2+IL7 stimulation, and 3) Human genome-wide transcriptome using the Illumina Platform compared in each HIV+ group to UI (FDR=0.05).

**Results:** HIV reservoirs were low (2 to 3 logcopies/10⁶ cells) with a comparable distribution among the 3 groups subsets and HIV-inducibility in all cases. The transcriptome analysis focusing on TCM, as main contributors to HIV reservoirs, individualized 3 major signatures compared to UI. The ARV-TCM, before or after IL-7: 1) shared gene modulation with EC-TCM, including a 2-fold PRDM-1/Blimp-1 over-expression, while 3-fold up-regulated in EC-TCM; 2) displayed a 2-3 fold augmentation of lymphocyte differentiation, cell death and transcriptional pathways including SATB1 (p=1.4x10⁻⁶, 9.2x10⁻⁷), the T lineage-restricted chromatin organizer and transcriptional repressor interacting with...
HIV-Tat. Third IL-7 increased transcription of activation pathways including the NR3C1 glucocorticoid-receptor proposed to regulate HIV provirus integration (p=9.2x10^-6).

Conclusions: Three key signatures relevant for silencing HIV reservoirs are revealed in CD4 central-memory T cells, a key HIV reservoir, from ARV-treated and EC subjects, involving distinct transcriptional repressors and regulators:
1. higher Blimp-1 upregulation among EC, part of an active immune and anti-inflammatory response;
2. a silencing SATB1-pathway signature associated with ARV and IL7;
3. an NR3C1-transrepressor signature as part of an IL7-induced inflammatory response, demonstrating that immune responses or therapeutic interventions dictate distinct mechanisms participating in in vivo regulation of HIV reservoirs.
Targeting and Eradication of Reservoirs

PE28 Transcriptomic and proteomic screening of host genes modulated by HIV-1 in human macrophages: a highly sensitive strategy for the identification of new targets specific of HIV reservoirs

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Background: Macrophages are among the first cells encountering the virus during the initial steps of the infection and can constitute a persisting virus reservoir. As antigen presenters and highly mobile cells, they can rapidly transfer the virus to other cells, enhancing the spreading of the infection to the entire immune system and accelerating disease progression. Nevertheless, very little is known about cellular events leading to successful virus replication in macrophages, due to the difficulty of studying separately productively-infected cells from “bystander” cells (uninfected and abortively infected cells not expressing HIV-1 proteins). We hypothesize that an efficient discrimination and isolation of these two populations, associated with a comparative transcriptomic/proteomic analysis will lead to the identification of several new key factors that can be targeted for the eradication of HIV reservoirs.

Methods: The Human Immuno-Retrovirology Laboratory (headed by Dr Michel J. Tremblay, Quebec City, Canada) recently designed a method allowing the separation of productively-infected and bystander cells through an immunomagnetic capture of the HSA tag-protein (Heat Stable Antigen) co-expressed with a fully replicative virus. We used this method to isolate infected MDMs from non-infected ones and analysed their transcriptomic and proteomic profile using a high-throughput RNA sequencing analysis and 2D-gel separation combined with mass spectrometry.

Results: This allowed us to detect with a high resolution the modulated transcripts and proteins controlling the successful replication of HIV-1 in macrophages. We identified several new genes, microRNA, long-non-coding RNA and proteins, as well as not- previously described cellular pathways that could be the keys for a specific eradication of infected cells in a mixed population. The different targets are now being tested by RNA interference for their functions susceptible to affect HIV-1 cycle, as well as those involved in macrophage physiology.

Conclusions: The acquired data will shed new light on HIV-1 replication mechanisms and allow the emergence of new specific inhibitory strategies against the constitution of persisting virus reservoirs and AIDS propagation. Proteins specifically expressed in infected cells are also interesting candidates for a vaccine targeting HIV-1 host cells instead of the virus himself that is known to be highly variable.
Immunotherapy depletion of T-cells in NHPs infected with SIV: impact on viral load and T-cell activation

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Background: There is considerable interest in the role of immune system ablation as a strategy to reduce the residual reservoir of HIV infection. One of the strategies that has been proposed is the use of T cell specific monoclonal antibodies. The theoretical concerns associated with this approach include the possibility of increasing viral replication or irreversible destruction of the immune system. In the present study we report the effects of an anti-monkey CD3 linked to diphtheria immunotoxin (C207) on 2 monkeys with treated SIV infection and one healthy control.

Methods: One healthy and two SIV-infected and treated (PMPA+FTC) rhesus macaques received intravenous injections of C207 over 2-4 days. Whole-body imaging of the CD4 pool using a $^{99m}$Tc radiotracer that specifically binds CD4 was performed on the healthy monkey at baseline and one week following the C207 injection; peripheral blood and axillary lymph nodes were analyzed for T and B cell phenotypes and rates of cell turnover. Levels of plasma and cell associated SIV-RNA and SIV-DNA were determined at similar time points.

Results: C207 induced 92-99% depletion of CD3+ cells in the blood. CD8+T cells (97-99%) were more profoundly depleted than CD4+T cells (88-98%). Whole body imaging of the CD4 pool revealed depletion of CD4+ T cells in the LNs and spleen. Flow cytometry demonstrated rapid increases in CD4 turnover in all 3 animals that were proportionate to total cell counts while CD8+ T cell turnover immediately increased and remained elevated. Plasma viral load and cell associated SIV-RNA and SIV-DNA remained below threshold of detection in both animals except for a single plasma viral blip of 90 copies/ml observed in one animal at day 2 post C207 injection. Following interruption of cART the viral load returned to pre-treatment levels in both animals.

Conclusions: T cell depletion with an anti-CD3 immunotoxin did not lead to a loss of control of viral replication or T cell homeostasis. Combining this strategy with other forms of immune-based therapies is currently being examined as a way to diminish viral reservoirs.
RNA targeting the promoter region potently inhibits HIV-1 activation from latently infected cells

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Background: We reported previously that certain RNA targeting the HIV-1 promoter region can induce transcriptional gene silencing (TGS), which is associated with heterochromatin formation in the HIV-1 promoter region to suppress HIV-1 transcription. We investigated whether or not the RNA based TGS approach is able to inhibit reactivation of HIV-1 in latently infected cells.

Methods: We transduced HIV-1 latently infected U1 cells, with a VSV-g pseudotyped lentiviral delivery system to express short-hairpin-RNA homologous to a conserved sequence in the HIV promoter (shPromA). We also transduced U1 cells with a 2-based mismatched variant (shPromA-M2), and scrambled (shPromA-Sc) as specificity control. These transduced U1 cells were then cultured in the presence of various stimuli, proinflammatory cytokine (TNF-α, 10ng/ml), histone deacetylase inhibitor (Trichostatin-A, 0.5µM), hematopoietic growth factor (GM-CSF, 1ng/ml) for 3 days. RT assays were used to determine the extent of newly related virus in the culture supernatants following activation.

Results: RT data indicated that massive amount of de-novo HIV-1 was produced from original latently infected U1 cells with TNF-α. RT levels were reduced by over 100 fold in culture supernatant of shPromA transduced-U1 cells, while shPromA-M2 transduced-U1 and shPromA-Sc transduced-U1 cells failed to suppress HIV-1 production from activated U1 cells. Similarly the shPromA transduced-U1 cells inhibited production of HIV-1 in the presence of other stimuli, Trichostatin-A and GM-CSF.

Conclusions: These findings suggested that shRNA targeting promoter region was able to maintain viral latency in U1 cells even in the presence of the strong activating stimuli. The induced suppression mediated by the shRNA was sequence specific. The shPromA seems to maintain the heterochromatin that characteristically associated with the promoter of the latent pro-virus preventing viral activation. TGS induced by shRNAs targeting the HIV-1 promoter region may be a useful strategy to sustain HIV latency and provide a pathway to a functional cure in future.
**Background:** The only adult considered cured of HIV infection received bone marrow transplantation (BMT), using Δ32 CCR5 donor marrow. Other patients receiving BMT plus continuous treatment with combination antiretroviral therapy showed substantial decreases in the latent reservoir. It is not completely clear why BMT eliminated or severely diminished the latent HIV reservoir. Only weak anti-HIV immune responses were observed following BMT. Another possible explanation for the latent reservoir depletion seen with BMT is that the conditioning regimens helped eliminate the reservoir, but while conditioning regimens certainly killed some, they probably did not kill all latently infected cells. We recently found that herpesviruses sense host cell apoptosis and respond by initiating an alternative, accelerated replication program. We hypothesized that a similar phenomenon could hold true for HIV: that latent HIV proviruses can also sense host cell apoptosis signals and respond by initiating replication in latent reservoir cells.

**Methods:** We studied several cell lines latently infected with HIV: ACH-2, J1.1, and U1. We treated the cell lines with agents that induce apoptosis, including 2[(3-(2,3-dichlorophenoxy)propyl)amino]ethanol (DCPE), and agents used in BMT conditioning regimens that induce apoptosis: etoposide, mycophenolate mofetil, and fludarabine phosphate. We treated the cells with caspase inhibitors in the presence or absence of apoptosis-inducing agents to test whether caspase activation was necessary for apoptosis-associated HIV activation. We assessed apoptosis flow cytometrically by Annexin V staining. We performed reverse transcription quantitative PCR on RNA from the treated cells to test for HIV activation and HIV p24 ELISA assays on supernatants from cells treated with apoptosis inducers to test for production of HIV protein.

**Results:** We found that apoptosis induction appeared to be associated with HIV activation. Apoptosis-mediated HIV activation was inhibited by caspase inhibitor, indicating involvement of caspases in HIV activation.

**Conclusions:** We conclude that, at least for some model HIV latently infected cell lines, initiation of apoptosis can activate HIV. The finding suggests that HIV can sense host cell apoptotic signals and respond by initiating replication. Understanding the HIV apoptosis sensing mechanism could yield new approaches to attack and deplete the latent HIV reservoir.
**Viral specificity of small molecule latency disruptors**

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**Background:** Eradication of the HIV reservoir may be accomplished by disruption of latency and consequent eradication of reactivated virus. Several clinical trials currently examine the efficacy of small molecules for this purpose. Similarly, an analogous approach has been attempted for years in the treatment of cancers driven by the Epstein-Barr virus (EBV). Given similarities in molecular mechanisms of latency between HIV and EBV, many compounds induce lytic replication in both viruses. Most AIDS survivors, unfortunately, suffer from co-infection of HIV and EBV. Increasing both viral loads may have adverse clinical consequences. We therefore tested whether mechanistically distinct classes of small molecules could disrupt either HIV or EBV latency specifically.

**Methods:** We asked if histone deacetylase inhibitors (SAHA and panobinostat), steroids (dexamethasone and prednisolone), DNA damage inducers (gemcitabine and bendamustine), and bromodomain inhibitors (JQ1 and I-BET) reactivate HIV and EBV in cell culture models of latency. Lytic replication was quantified with flow cytometry by measuring an immediately early protein of EBV and a reporter protein of recombinant HIV.

**Results:** Histone deacetylase inhibitors disrupt latency of both HIV and EBV. Steroids and DNA damage inducers only reactivate EBV. Bromodomain inhibitors only reactivate HIV.

**Conclusions:** We find that certain classes of small molecules specifically disrupt latency of only either HIV or EBV, while some compounds promote reactivation of both viruses. Such virus-specific latency disruptors may be preferred in therapeutic strategies. We therefore advocate that bromodomain inhibitors be examined as an alternative to histone deacetylase inhibitors in the eradication of HIV reservoirs.
A dual-reporter cell line, expressing basal levels of Tat from its native IRES, reveals the poor specificity of current epigenetic modifying drugs for proviral HIV-1 activation

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Background: The persistent reservoir of latently infected CD4⁺ T cells necessitates lifelong combination anti-retroviral therapy and is linked to chronic immune activation and serious comorbidities driven by HIV products such as viral-RNA or Tat. Epigenetic modifying drugs are under investigation to purge the latent reservoir by specifically inducing expression from the integrated provirus, while avoiding global cell activation, but their specificity in cells expressing HIV RNA or Tat is unclear.

Methods: We generated a cell line containing a sub-genomic reporter provirus (LTR-nef3/Click-Beetle-Red-Luciferase), exhibiting minimal reporter expression in the absence of Tat, but is highly responsive to Tat over a wide dynamic range. Low-level Tat expression was stably introduced by transducing cells with the first t coding exon, constructed to depend on the native Internal Ribosome Entry Site (IRES) within the tat exon for expression. This recapitulates Tat-expression from cellular-provirus read-through transcripts that were identified in HIV latent cells. The cells also contain a Click-Beetle-Green-Luciferase, driven by the Tat-independent CMV-IE promoter, allowing quantification of non-specific gene-activating effects. This cell line was used to evaluate the HIV-specificity of published epigenetic modifiers such as JQ1 and SAHA, and to screen a lead-like small molecule library.

Results: Known epigenetic drugs and ~114,000 lead-like compounds were evaluated in high throughput screens, quantifying the HIV specific (LTR-Red) gene expression relative to non-specific (CMV-Green) gene expression. As expected, transfected recombinant Tat protein controls demonstrated potent specific LTR activation, (at ~175pM), and no associated change to the CMV promoter. Epigenetic modifying drugs currently undergoing clinical investigation, including the HDAC-inhibitor SAHA and the BET-bromodomain inhibitor JQ1, demonstrated moderate to high levels of LTR activation, however, this was also associated high levels of undesirable non-specific CMV promoter activation. Importantly, screening has also identified several novel small molecules that act very specifically on the LTR, while avoiding CMV activation.

Conclusions: We have developed a rigorous model for high throughput detection of novel drug candidates that specifically modulate HIV provirus on the background of low intrinsic expression of some HIV products (viral RNA and Tat). We are now developing the novel small molecule leads to iteratively enhance their HIV-1 specificity and potency.
Modeling the effects of vorinostat in vivo on activation of latent HIV-infection

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Background: Long lived latently infected resting memory T-cells that persist in HIV-infected patients on combination antiretroviral therapy (ART) are the major barrier to cure. Activation of latent infection may be a potential strategy to eliminate these long lived cells. The histone deacetylase inhibitor (HDACi), vorinostat, has been shown to activate transcription of HIV RNA in latently infected cells in vitro and in vivo. We recently demonstrated that vorinostat given once daily for 14 days to HIV-infected patients on ART, induced a significant and sustained increases in cell associated unspliced (CA-US) HIV RNA in the majority of patients, however, there was no change in HIV DNA. The goal of this study was to develop a mathematical model that could accurately fit the change in CA-US HIV RNA following vorinostat.

Methods: HIV-infected adults on suppressive cART (n=20) were enrolled in a prospective single arm study and received vorinostat 400 mg once daily for 14 days. Blood was collected at 0, 2, 8 and 24 hours, and 7, 14, 21, 28 and 84 days. CA-US HIV RNA was quantified in CD4+ T-cells from blood.

Results: We constructed viral dynamic models that included latently infected cells and incorporated the effects of vorinostat treatment. We developed two models - one that assumed a single type of latently infected cell (model A) and a second model (model B) by adding another type of latently infected cell that was activated more slowly than the cells in model A. The model with two latently infected cell populations, one that was rapidly-reactivated (within one day of treatment) and one that was slowly-reactivated upon treatment, fit the data better than a model with a single latently infected cell population. Fitting the model to changes in CA-HIV RNA further suggested that vorinostat treatment (in conjunction with cART) may also reduce the size of the latent reservoir.

Conclusions: The variable kinetic changes in CA-US HIV RNA following vorinostat may potentially be explained by two populations of latently infected cells, which may reflect a ‘deeply latent’ cell subpopulation in the reservoir.
**PE35 Development of T-pharmacytes for the targeted eradication of HIV reservoirs**

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**Background:** The purging of HIV reservoirs will likely require a “flush-and-kill” approach, combining delivery of a latency reversing drug with effective immune clearance. To this end, we have developed a means of stably attaching drug-loaded nanoparticles (NPs) to cytotoxic T-cells (CTL) surfaces, converting them into living carriers of therapeutic cargos. These ‘T-Pharmacytes’ effectively lyse target cells, traffic normally in vivo, and specifically deliver their cargo to sites of antigen expression. In prior work, coupling of NPs carrying IL-15 superagonist (IL-15SA) to antigen-specific T-Pharmacytes dramatically enhanced tumor eradication in a murine melanoma model as a result of enhanced CTL function. Since we have found that IL-15SA is also a potent HIV latency reversing drug, we propose that IL-15SA loaded T-Pharmacytes could integrate the “flush” with the “kill” by delivering IL-15SA to lymphoid tissue, reactivating latently-infected cells, and then eliminating these unmasked targets.

**Methods:** Lipid-based nanoparticles were loaded with the human IL-15SA ALT-803 and conjugated to either HIV-specific or CMV-specific CTL. The killing of target cells and release of fluorescently labeled cargo was observed by time-lapse microscopy. T-Pharmacytes loaded with the IL-15SA ALT-803 were co-cultured with primary cell models of HIV latency and latency-reactivation was measured by luciferase expression, or by CTL recognition of targets. Adoptive transfers of unmodified or ALT-803-loaded T-Pharmacyte were performed in xenograft mouse models of HIV infection.

**Results:** Recognition of peptide-pulsed CD4⁺ T-cells by HIV-specific T-Pharmacytes resulted in efficient killing of these targets, as well as lysis of the lipid NPs and burst release of NP cargo. ALT-803-loaded T-Pharmacytes reversed HIV latency in trans, permitting recognition of reactivated targets by CTL. Preliminary data from a humanized mouse model of HIV-infection supports suggests in vivo anti-viral activity of T-Pharmacytes, with adoptive transfer of ALT-803-loaded HIV-Gag-specific T-Pharmacytes resulting in 2-3 log reductions in viral load for 65 days. Rebound viremia from natural HIV reservoirs was consistently observed in NSG mice reconstituted with cells from ARV-treated patients, providing a model for future testing of the abilities of autologous HIV-specific T-Pharmacytes to eradicate natural viral reservoirs.

**Conclusions:** Our results thus far encourage further development of T-Pharmacytes as an integrated flush-and-kill therapeutic to eradicate persistent HIV reservoirs.
PE36 Quantification of drug transporters in vaginal and cervical tissue using a novel targeted proteomics approach: implications for small molecule disposition in viral reservoirs

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Background: The female genital tract (FGT) has been identified as a potential viral reservoir. Successful HIV eradication will require adequate antiretroviral and/or latency-reversing agent concentrations in this compartment. Identifying the transporters governing drug disposition would greatly inform the development of targeted HIV therapies for treatment and cure. Limitations exist in characterizing drug transporter mRNA (due to post-transcriptional modifications that hinder protein translation) and protein expression (due to antibody cross-reactivity). We explored a novel proteomics approach and hypothesize that absolute quantification will provide more accurate representations of transporter expression, and better inter-tissue comparisons.

Methods: Pathologically normal cervical (n=10) and vaginal (n=10) tissues collected from gynecologic surgeries were snap frozen and subsequently homogenized prior to membrane fraction isolation. Nineteen transporters were evaluated based on antiretroviral affinity. A Waters NanoAcquity LC coupled to an AB SCIEX QTRAP 5500 was used for analysis. Concentrations were calculated relative to heavy labeled peptides. Two liver samples were analyzed for comparison. Paired t-tests or Mann-Whitney U tests were used to compare transporter concentrations between tissues. Data are reported as mean ± standard deviation.

Results: Quantifiable concentrations were observed for 1 efflux (MDR1; 0.3 ± 0.2 pmol/mg protein and 0.7 ± 0.7 pmol/mg protein in cervical and vaginal tissue, respectively) and 2 uptake (OCT3; 7.7 ± 4.5 pmol/mg protein and 25.5 ± 11.2 pmol/mg protein, OATP2A1; 0.7 ± 0.3 pmol/mg protein and 1.0 ± 0.4 pmol/mg protein) transporters. Higher OCT3 concentrations were observed in vaginal tissue versus cervical tissue (p< 0.001). Cervical and vaginal transporter concentrations were 15 and 32% of liver expression for MDR1, 78 and 259% for OCT3, and 1110 and 1647% for OATP2A1, respectively.

Conclusions: We quantified 3 drug transporters in FGT, which is consistent with previously published mRNA data. However, we were unable to detect several transporters that have been previously identified by mRNA. Since our method directly measured protein concentrations, it better represents in vivo transporter expression and focuses on those of most importance for drug disposition. Comparison to liver tissue suggests that the observed concentrations have biological relevance. Ultimately, these data will inform the design of targeted therapies towards elimination of the HIV reservoir.
THE 'KICK-AND-KILL' APPROACH TO A CURE: GOING FOR THE KILL BY SELECTIVE INDUCTION OF APOPTOSIS IN LATENTLY INFECTED CELLS

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BACKGROUND: Cells latently infected with HIV-1 persist for decades, constituting a major barrier to curing AIDS. The ‘kick-and-kill’ strategy is premised on pharmacological reactivation of virus production in such cells (‘kick’), leading to their death by intrinsic or extrinsic biological mechanisms (‘kill’). However, cells may persist once driven into HIV-1 production. Whereas numerous agents have been explored as reactivators of latently infected cells, drugs that selectively ablate HIV-infected cells have received less attention. The drugs deferiprone and ciclopirox cause selective death of HIV-infected cells in vitro by therapeutic reclamation of apoptotic proficiency (TRAP). Both drugs inhibit HIV-1 gene expression and replication without on-drug breakthrough or off-drug rebound (Hanauske-Abel et al. (2013) PLoS One 8: e74414). In a proof-of-concept clinical trial, deferiprone decreased the viral load of individuals who achieved a preset threshold drug concentration in blood, and HIV-1 did not rebound off-drug when monitored for >6 weeks (~670 times the deferiprone half-life) (in preparation). To determine whether TRAP might be harnessed for the ‘kill’ requirement, we examined the ability of deferiprone and ciclopirox to trigger apoptosis in a model of latent infection.

METHODS: RLatently infected Jurkat 3C9 cells and uninfected Jurkat E6 cells were stimulated with TNF-α and treated with 200 µM deferiprone or 5 µM ciclopirox. Live and dead cells, apoptosis (Annexin V, caspase-3), and HIV induction (EGFP reporter) were quantified by FACS analysis.

RESULTS: Deferiprone increased cell death in latently infected, TNF-stimulated cells, and to a lesser extent in unstimulated cells. Active caspase-3 was demonstrated in dying EGFP-expressing cells. Only modest cell death was seen in HIV-uninfected cells. Similar data were obtained with ciclopirox. The results indicate that these drugs trigger cell death via TRAP in latently HIV-infected Jurkat cells, especially after HIV-1 reactivation.

CONCLUSIONS: The drugs deferiprone and ciclopirox are pioneer ablative anti(retro)virals capable of destroying latent and active viral production sites, and may have application in eradicating latently infected cells. Our findings raise the prospect of ablating the latent reservoir either by (1) treatment with TRAP-inducing drugs alone, if reservoir cells are sufficiently sensitive in vivo; or (2) a combination of HIV-1 reactivators and TRAP inducers.
Multidose vorinostat in HIV-infected individuals on effective ART leads to an increase in regulatory T-cells but no change in inducible virus or HIV-specific T-cells

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Background: Histone deacetylase inhibitors (HDACi) activate HIV transcription in latently infected T-cells in vitro and in HIV infected subjects on suppressive antiretroviral therapy (ART). HDACi have also been reported to have widespread largely suppressive effects on both the innate and adaptive immune response. This study aimed to examine whether administration of 14 days of vorinostat to HIV-infected patients on ART led to a change in inducible virus or adaptive immune response.

Methods: Vorinostat 400mg orally was given daily for 14 days to 20 HIV-infected individuals on ART. Staphylococcal enterotoxin B(SEB) and HIV-gag specific T-cells were measured using intracellular cytokine staining for interleukin(IL)-2, tumor necrosis factor (TNF)-α and interferon (IFN)γ (n=17). Intracellular FoxP3 staining was used to determine percentage of regulatory T-cells (Treg). Integrated HIV-DNA in CD4 T-cells was quantified by nested real time PCR at days 0, 14 and 84. Inducible reservoir was quantified by tat-rev inducible limiting dilution assay (TILDA) in CD4 T-cells stimulated with phorbol myristate acetate (PMA) and ionomycin prior to and following vorinostat. The frequency of positive cells detected by qPCR for tat-rev multiply spliced (MS)-RNA was calculated using the maximum likelihood method. Significant changes over time were determined by generalised estimating equation (GEE).

Results: There was a significant increase in CD4+ Treg cell at day 14 (p=0.046) which returned to baseline by day 84. SEB-specific CD8 T-cells that produced IFN-γ also increased significantly during and following vorinostat (p=0.026) while there were no significant changes in other SEB-specific CD8+ T-cells and all SEB-specific CD4+ T-cells. There were no significant changes in gag-specific CD4 or CD8 T-cells. We saw no significant change in integrated DNA (n=11) or the frequency of CD4+ T-cells that produced msHIV RNA following mitogen stimulation measured by TILDA (n=7) compared to baseline indicating no change in inducible virus from the latent reservoir following vorinostat.

Conclusions: Administration of vorinostat to HIV-infected patients on ART led to significant potentially adverse immunological changes including an increase in T-regs without any significant change in HIV-specific T-cells. Future strategies to reduce the latent reservoir will require more potent latency reactivating agents and likely combination with immune modulators that boost HIV-specific immunity.
Host Genetics of Resistance and Susceptibility

**PE39** Evolutionary analysis identifies an MX2 haplotype associated with natural resistance to HIV-1 infection

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**Background:** The protein product of the human MX2 (myxovirus resistance 2) gene was recently shown to act as a post-entry inhibitor of HIV-1 infection. MX2 and its paralog MX1 belong to the dynamin-like large GTPase superfamily and, whereas MX1 can restrict a wide variety of viruses, MX2 is known to counteract retroviruses only. The human and macaque MX2 proteins efficiently restrict HIV-1 and other simian immunodeficiency viruses, but have a modest effect against retroviruses that infect non-primate species. This observation points to species-specific virus-host interactions that may result from evolutionary arms races. This research project, thus, aimed to demonstrate the relevance of MX2 variants to HIV-1 infection susceptibility in human populations.

**Methods:** We applied a multidisciplinary approach to study:
1. evolutionary history of MX2 in placental mammals (PAML analysis);
2. structural models of MX2;
3. genotyping analysis of rs2074560 in the MX2 gene in 3 independent case-control cohorts of HIV-1 exposed seronegative individuals (HESN); and
4. in vitro HIV-1 replication plus IFNα-stimulated MX2 mRNA expression in peripheral blood mononuclear cells (PBMC) from healthy controls (HC) grouped according to their MX2 genotype.

**Results:** MX2 evolved adaptively in mammals with distinct sites representing selection targets in rodents and primates; pervasive selection mainly involves residues in loop 4, previously shown to carry antiviral determinants. We also demonstrated that natural selection operated on MX2 during the recent history of human populations: distinct selective events have driven the frequency increase of two haplotypes in populations of Asian and European ancestry. The Asian haplotype carries a susceptibility
allele for melanoma; the European haplotype is tagged by rs2074560, an intronic variant. By analysis of three independent HESN cohorts with different geographic origin and exposure route we verified that the ancestral (G) allele of rs2074560 protects from HIV-1 infection with a recessive effect (combined p value of 1.55x10^{-4}). In line with these findings, the G allele is associated with lower in vitro HIV-1 replication and increased MX2 expression.

Conclusions: Results herein establish a role for MX2 as a central element of antiviral response in mammalian species and a possible target for therapeutic intervention in HIV-1 treatment and prevention.
Host Restriction Factors (Including APOBEC and TRIM)

**PE40 Quantification of host restriction factor gene expression in HLA-B*57+ individuals**

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**Background:** HLA-B*57 stands out as a major allele associated with lower viral loads. However, this genetic trait only accounts for about 25% of the variance in HIV-1 viral load. It is not clear why some HLA-B*57+ individuals progress more rapidly to disease compared to others who also have the allele, but are slow progressors. We previously reported that PBMC from HLA-B*57+, but HIV-1-seronegative individuals have increased expression levels of APOBEC3A, APOBEC3B, BST-2/tetherin and ISG15, and CD4+ T cell activation levels strongly correlated with restriction factor expression (Raposo RA, J Leukoc Biol, 2013). Therefore, we hypothesized that host restriction factors may play a role in disease progression in HLA-B*57+ individuals. In this study, we comprehensively analyzed the restriction factor gene expression profile of HLA-B*57+ individuals over the course of disease.

**Methods:** We quantified restriction factor gene expression in PBMC from six HLA-B*5701+ HIV-1-positive MSM enrolled in the Options cohort at UCSF over 8 years. Patients were divided into 2 groups based on CD4 counts at baseline, shown to predict the risk of progression to AIDS. We quantified CD4+ T cell activation levels by cytometry and we developed a custom real-time PCR array to measure the mRNA expression of 34 established anti-HIV-1 restriction factors. To represent overall cellular anti-HIV-1 restriction capacity, we defined a CuRe (Cumulative Restriction) score as cumulative fold-difference in restriction factor expression with respect to a control individual. Unpaired t tests were used to compare gene expression levels and correlations were analyzed using Spearman’s rank coefficient.

**Results:** Levels of CD4+ T cell activation and CuRe scores were significantly elevated in high-risk progressors early after infection (p< 0.05) and correlated with a decline in CD4 counts (Spearman R=-0.92, P< 0.0001). In contrast, low-risk progressors had lower levels of CD4+ T cell activation at early stages of infection, and had lower CuRe scores, which gradually increased over time. This pattern likely reflects a scenario in which restriction factor expression is primarily driven by cellular activation and interferon exposure in vivo.

**Conclusions:** We show that combining an anti-HIV-1 restriction factor gene expression array and immunological parameters provide important measures of disease progression in HLA-B*57+ individuals.
Therapeutic Vaccines

PE41 Therapeutic vaccine-induced Gag-specific CD8+ T cells under anti-retroviral therapy contribute to viral control in a macaque AIDS model

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Background: Antiretroviral therapy (ART) can inhibit HIV replication but not eradicate viruses from HIV-infected individuals. Because ART-based HIV control is often accompanied by reduction in virus-specific CD8+ T cell responses, enhancement of these responses may contribute to more stable viral control under ART. In the present study, we examined the impact of virus-specific CD8+ T cells induced by therapeutic vaccination under ART on viral control in a macaque AIDS model.

Methods: Twelve Burmese rhesus macaques received ART from week 12 to 32 after SIVmac239 challenge. Six of them were intranasally vaccinated with Sendai virus vectors expressing Gag and Vif (SeV-Gag and SeV-Vif) at weeks 26 and 32. We examined plasma viral loads, antigen-specific CD8+ T cell responses, and in vitro capacity of CD8+ cells at weeks 10 (pre-ART), 27 (during ART), and 34 (post-ART) to control replication of SIVs derived from PBMCs at weeks 10 (pre-ART SIV) and 34 (post-ART SIV), respectively.

Results: All macaques showed persistent SIV infection, but controlled viremia after the start of ART at week 12. After ART cessation at week 32, reappearance of plasma viremia was observed in all, but vaccinees showed significant reduction in rebound viral loads compared to those in unvaccinated macaques. While SIV-specific CD8+ T cell responses induced before ART start were reduced during ART, the vaccination resulted in efficient Gag/Vif-specific CD8+ T cell responses. Vaccinated animals had CD8+ cells at week 27, 1 week after the first vaccination, with higher anti-SIV capacity than pre-ART (at week 10). The capacity of these CD8+ cells to control pre-ART SIV replication was associated with Gag-specific CD8+ T cell responses at week 27. Furthermore, the capacity of post-ART CD8+ cells to control post-ART SIV replication was associated with Gag-specific CD8+ T cell responses at week 34. This anti-post-ART SIV capacity of post-ART CD8+ cells inversely correlated with rebound viral loads after ART cessation. These imply that Gag-specific CD8+ T cell induction by therapeutic vaccination results in reinforcement of CD8+ cell capacity to control SIV replication.

Conclusions: Our results suggest that therapeutic vaccine-induced Gag-specific CD8+ T cells can contribute to HIV control.
Heteroclitic human immunodeficiency virus peptides augment cytokine production, increase proliferation and reduce PD-1 expression by HIV-specific CD8+ T-cells

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Background: Human immunodeficiency virus (HIV) infection often leads to decreased T cell cytokine production, decreased proliferation and ultimately apoptosis of HIV-specific CD8+ T cells. Immunotherapy for HIV infection should address this “exhaustion” of HIV-specific memory CD8+ T cells and efficiently activate potent HIV-specific T cells from naïve T cells. Heteroclitic peptides are sequence variants of native peptide epitopes that stimulate better T cell responses than the native epitope. We previously demonstrated that heteroclitic peptides enhance interferon-gamma (IFN-γ) and interleukin-2 (IL-2) production by HIV-specific CD8+ T cells. In this study, we tested whether they can also enhance CD8+ T cell proliferation and reduce signs of exhaustion.

Methods: Twenty-four variant peptides were synthesized based on reference human histocompatibility-linked leukocyte antigen (HLA)-A2-restricted HIV peptide epitopes Nef 83→91, Nef 135→143, Gag 77→85 and Gag 433→440 with conservative and semi-conservative amino acid substitutions at positions 3, 5 or 7 of the first three and 3, 5 or 8 of Gag 433→440. Heteroclitic peptides were identified in ELISPOT assays with peripheral blood mononuclear cells (PBMC) from 25 HIV-infected HLA-A2 individuals by stimulation of ≥100 more IFN-γ sfu/106 PBMC or 50 more IL-2 sfu/106 PBMC than the reference peptide. Forty-one instances of heteroclitic peptide activity were observed by ELISPOT. Heteroclitic peptides were then used to stimulate the same donor’s PBMC in cell culture. After 7 days, total CD8+ T cell proliferation and the percentage of proliferating cells expressing programmed death-1 (PD-1) were assessed relative to stimulation with reference peptides.

Results: Heteroclitic peptides augmented HIV-specific CD8+ T cell proliferation by >20% in 13 cases and stimulated similar proliferative responses to reference peptides in 17 cases. In addition, heteroclitic peptides reduced PD-1 expression by 15%-50% in 10 cases, and by ≥50% in 4 cases.

Conclusions: Heteroclitic peptides elicit stronger HIV-specific CD8+ T cell cytokine and proliferative responses relative to reference peptides and modulate the differentiation status of responding cells, indicating their unique immunotherapeutic potential.
Allogeneic assassination: NK cells demonstrate robust anti-HIV antibody-dependent activation against allogeneic targets

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Background: Cell-to-cell HIV transmission is more efficacious than virion-to-cell transmission in vitro and in vivo. Despite evidence of allogeneic HIV-infected “Trojan horse” cells initiating HIV transmission through cell-to-cell infection, many current vaccine constructs aim to neutralize free virions. RV144 analyses have highlighted an association between antibody-dependent cytotoxicity, which is mediated by NK cells, and vaccine efficacy. Epidemiological research suggests NK cells can prevent HIV transmission in donors co-carrying allelic combinations of the inhibitory KIR3DL1 and its HLA-Bw4 ligand, which confer NK cells with enhanced functional potential. Given the need for HIV vaccines to eliminate HIV-infected allogeneic cells, we investigated whether NK cells exhibit anti-HIV antibody-dependent activation against allogeneic targets.

Methods: TT-lymphocytes enriched via magnetic selection from lymphocytes of two donors, which expressed or lacked HLA-Bw4, were cultured overnight in IL-2 supplemented media. Next, T-lymphocytes were coated with gp120. Coated and uncoated T-lymphocytes were incubated with whole blood from nine donors with KIR3DL1⁺ NK cells in the presence of HIV⁺ or HIV⁻ plasma. Fluorochrome-conjugated antibodies against CD3, CD56, and KIR3DL1 were used to identify NK cells, and antibodies against CD107a and IFNγ detected activation.

Results: NK cells demonstrated robust anti-HIV antibody-dependent activation in response to gp120-coated allogeneic T-lymphocytes (Median(Range) % activated NK cells; 15.5%(0-30.8%)). Higher proportions of educated KIR3DL1⁺ NK cells from HLA-Bw4⁺ donors exhibited activation against allogeneic targets than KIR3DL1⁻ NK cells (30.2%(5-60.9) Vs. 12.0(2.8-25.9), p< 0.05). Lastly, KIR3DL1⁺ NK cells were not inhibited by the presence of HLA-Bw4 on target cells, as the percent contribution of KIR3DL1⁺ NK cells to the total NK cell response was not lower against HLA-Bw4⁺ targets as compared to HLA-Bw4⁻ targets.

Conclusions: NK cells exhibit anti-HIV antibody dependent activation against HIV antigen-expressing foreign lymphocytes. This provides a mechanism to understand antibody-dependent NK cell-mediated protection from HIV transmitted by infected cells. Further, NK cells can overcome inhibition by KIR3DL1/Bw4 interaction to still mediate anti-HIV antibody-dependent functions. Future research elucidating the mechanism(s) involved in overcoming inhibition through KIR/HLA interactions will allow design of vaccines utilizing optimized NK cell antibody-dependent responses to eliminate allogeneic HIV-infected lymphocytes.
Impact of RNA loaded Dendritic cell immunotherapy on HIV sequence Evolution in Chronic HIV Subjects Undergoing ATI

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Background: AGS-004 is a personalized immunotherapy consisting of dendritic cells (DCs) loaded with RNA encoding autologous Gag, Nef, VPR, and Rev. Previously we reported clinical study results of a Phase 2a clinical trial designed to assess the impact of AGS-004 during a 12 Week ART analytical treatment interruption (ATI) in chronic HIV-1-infected subjects. In this follow up study we compared plasma HIV sequences before initiation of ART to after AGS-004 treatment during ATI.

Methods: Plasma samples were collected prior to ART (before AGS-004 treatment) and at first peak of viral rebound during ATI. Samples from four subjects were analyzed. One subject maintained undetectable plasma VL post ATI for 153 days (controller), whereas three other subjects had detectable viremia within 12-14 days post ATI (non-controllers). Viral sequences corresponding to the full-length immunogens were cloned and analyzed by Sanger sequencing. Sequences were aligned in MEGA software using clustalW to construct phylogenetic trees using maximum likelihood and evolutionary divergence was calculated using the Tajima Nei method.

Results: Phylogenetic tree analyses revealed a clear shift in all four subjects’ sequences from pre-ART, pre-AGS-004 treatment to post-viral rebound during ATI. The controller subject had the lowest divergence change in all sequences from pre ART to the ATI time point compared to non-controllers. The time from first detectable virus in plasma to first VL peak in the controller subject was 54 days and 17-27 days for the non-controllers. The VL reduction from pre-ART set point to first peak was also greatest in the controller subject (0.9 log10 verses -0.2-0.4 log10 for non-controllers). Changes in CTL epitopes relevant to HLA haplotypes were also identified.

Conclusions: The combination of sequence changes corresponding to AGS-004 targets, highest decrease in VL post-ATI, longest time to detectable virus post-ATI and lowest divergence suggest that AGS-004-induced immunological pressure in the controller subject impacted viral evolution. To our knowledge this is the first report associating HIV antigen sequence divergence in response to autologous therapeutic immunization with durability of VL control. The role AGS-004 plays in the induction of viral evolution is being further elucidated in the double blinded placebo-controlled phase 2b clinical trial.
Use of a new, transmitted founder SIV Strain (SIVsmmFTq) to achieve control of virus replication with conventional antiretroviral therapy (ART) in rhesus macaques (RM)

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**Background:** Animal models for cure research are badly needed, but SIV infection in RM is very difficult to control with ART. We identified SIVsmm strains that more closely reproduce in RM the pathogenesis of HIV-1. We hypothesized that these new strains can be more readily controlled with conventional ART regimen than the reference SIVmac strains.

**Methods:** Nine RMs (MaMu A*01, B*08, B*17 neg and Trim5a matched) were iv-infected with the SIVsmmFTq transmitted-founder infectious molecular clone. Four RMs received ART (PMPA, 20 mg/kg/day; FTC, 40 mg/kg/day; integrase inhibitor L870812, 20 mg/kg bid) for 9 months starting from 65 days postinfection (dpi). Viral replication was assessed by monitoring plasma viral load (VL) with conventional and single copy assays. Proviral DNA 2-LTR circles and cell-associated RNA were monitored. Changes in the memory CD4+ T cells in the intestine, T cell immune activation and proliferation, and biomarkers associated with disease progression in RMs (C-reactive protein, D-dimer, IL-6 and sCD14) were assessed.

**Results:** SIVsmmFTq VL peaked at 7-8 logs/ml at 10 dpi and reached the viral set-point (4.2-5.4 logs/ml) by 42 dpi. After initiation of ART, VL decreased by 1.5-2 logs in 48 hours, followed by a slower decline to undetectable levels (< 5-10 copies/ml) that were reached by 30 days post-treatment initiation. Over the next 8 months, VLS remained undetectable (< 100 copies/ml when VLS were assessed with the conventional assay and < 10 copies/ml when assessed with the single copy assay), without blips. Restoration of mucosal memory CD4+ T cells and reduction in immune activation occurred in ART-receiving RMs but not in untreated controls. ART also normalized biomarkers associated with disease progression and mortality in RMs.

**Conclusions:** We established a RM model of viral suppression using a conventional ARV regimen analogous to current ART employed for HIV-1 infection. With this regimen, suppression is robust, with no blip of viral replication over an 8 month period. We established the virologic monitoring capacity of SIVsmmFTq. This simplified animal model of ART enables the investigation of the distribution and evolution of viral reservoir under ART as well as evaluation of therapeutic interventions aimed at eliminating the persistent viral reservoir.
Immune-Based Therapy Trial

PE46 A pilot study of the impact of rosuvastatin administration on residual chronic immune activation under antiretroviral therapy: the CESAR-IMEA trial

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Background: Residual chronic immune activation in treated patients is associated with incomplete CD4 cell recovery and increased morbidity/mortality. Statins were shown to exert anti-inflammatory properties independent of changes in serum cholesterol. The aim of the trial was to evaluate, in patients receiving effective ART, the effect of addition of rosuvastatin on cellular and soluble markers of immune activation.

Methods: CESAR was a Phase II open-label pilot bicentric trial that enrolled patients under cART for at least 24 months with plasma VL< 40c/mL, CD4 < 500/mm3, CRP< 10mg/L and no indication for a statin treatment. Patients received rosuvastatin (20mg/d) for 3 months and were followed-up 3 additional months. The primary outcome was the variation at week 12 (W12) in the proportion of CD38+HLA-DR+CD8+ T lymphocytes. Secondary outcomes included evolution of CD8 and CD4 T-cell activation and of soluble inflammatory biomarkers between baseline (BL), W12 and W24. CRP was analyzed only in patients with CRP< 10 mg/L.

Results: 50 patients were enrolled, 45 completed the study, endpoints were available for n=43. Median (IQR) BL CD4 counts were 319 (284-442). The proportion of CD38+HLA-DR+CD8+ T cells did not decline significantly throughout the follow-up. However, the proportion of CD38+CD8+ T cells significantly decreased at W12 and the decrease was sustained until W24. The % Ki67+CD4+ T cells significantly decreased at W12. CRP levels declined significantly between BL and W12 (p= 0.047) whereas levels of IL-6, sCD14 and D-dimer did not change. Serum total and LDL-cholesterol significantly decreased at W12 (p< 0.001) and returned to BL values at W24.
Conclusions: Addition of rosuvastatin to effective ART regimen resulted in a sustained decrease in CD8 T cell activation. Further trials are warranted to confirm these promising results.
Eradication / Reservoir Depletion: Impact of ART and Non-ART Treatment

**PE47 LB Impact of Early Initiation of Combination Antiretroviral Therapy on Measures of Virus in Peripheral Blood of Vertically HIV-1-Infected Children**

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**Background:** Triple combination antiretroviral therapy (cART) as HIV-post exposure prophylaxis is routinely administered to newborns at high risk for HIV infection in our clinical centres. We investigated measures of HIV-1 reservoir size in peripheral blood of HIV-1-infected children with sustained virologic suppression (SVS) following initiation of cART within 72 hours of birth.

**Methods:** Health records of children born to HIV-1-infected mothers started on cART within 72 hours of birth were reviewed. Those with HIV-1 infection and SVS underwent further investigation, including HIV-1 serology, HIV-1-specific cell-mediated responses, HLA genotyping, ultra-sensitive viral load (VL), and measurement of cell-associated HIV-1 DNA and RNA and replication-competent HIV in peripheral blood. SVS was defined by the absence of detectable virus in standard VL assay subsequent to achieving an undetectable VL (< 50 copies/mL) for the first time.

**Results:** 136 newborns initiated cART within 48 hours of birth (ZDV/3TC/nevirapine (n=56); ZDV/3TC/nelfinavir (n=73); ZDV/3TC/lopinavir/r (n=7)). Twelve of 136 were vertically infected (8.8%). In 6 of 12, no consistent suppression of VL was achieved due to poor adherence. Two children achieved and maintained an undetectable VL for 2-3 years, but experienced virologic rebound subsequent to poor adherence. In the four children with SVS, HIV serology, HIV-specific cell-mediated immune responses (Nef, Gag) and ultrasensitive VL were negative. Proviral DNA was not found in enriched CD4⁺ T-cells (< 2.6 copies/mg DNA), whereas HIV RNA was detected in all four children (19.5-130 copies/1.5 mg RNA). No virion-associated HIV RNA was detected following mitogenic stimulation (5.4-8.0 million CD4⁺ T-cells) in these four children, but replication competent virus was detected by quantitative co-culture involving a higher number of cells in one (0.1 infectious units/million CD4⁺ T-cells). HLA B*58 and HLA-B sequence variations associated with better HIV control were found in 3 of the 4 children.
Conclusions: Absence of detectable HIV-1 DNA and absence or low levels of replication-competent virus in peripheral blood in a subgroup of HIV-1 infected children initiated on cART within 72 hours of birth suggests that early cART could significantly reduce HIV reservoir size. Cessation of cART may be necessary to determine whether functional HIV cure can be achieved in such children.
PE48 LB  Allogeneic bone marrow transplantation in two HIV-1 infected patients shows no detectable HIV-1 RNA or DNA, and a profound reduction in HIV-1 antibodies

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Background: Allogeneic bone marrow transplantation (BMT) can have significant effects on viral reservoirs in HIV-1 infected individuals, and in one case led to an apparent sterilising cure.

Methods: We studied two HIV-1 infected patients who had undergone allogeneic BMT with reduced intensity conditioning (RIC) for haematologic malignancies. HIV-1 antigens and antibodies (Ag/Ab) were measured by 4th generation chemiluminescence microparticle immunoassay (CMIA) and by Western blot (WB). HIV-1 specific CD4+ T cell responses were measured by CD25/CD134 upregulation. HIV-1 RNA levels in plasma were measured by two separate real-time PCR assays with 20 as well as single copy/ml sensitivity; HIV-1 DNA levels were assessed in peripheral blood mononuclear cells (PBMCs) as well as in isolated CD4+ T cells by PCR using three different primer sets. Both patients were tested for the presence of the CCR5Δ32 mutation by PCR.

Results: Two subjects (A and B) received HLA matched, allogeneic stem cell transplants in 2010 (A) and 2011 (B) for non-Hodgkin’s lymphoma and acute myeloid leukaemia respectively. Both patients remained on antiretroviral therapy during and following the procedure. Post-transplant, subject A experienced systemic grade 2 graft versus host disease (GVHD), whereas subject B developed only mild, skin related GVHD. Patient A was heterozygous for the CCR5Δ32 mutation post- transplant, patient B was CCR5 wildtype. Following BMT, both patients had no detectable HIV-1 RNA in plasma by either real-time PCR assay, and no detectable HIV-1 DNA by PCR in PBMCs or CD4+ T cells. CD4+ T cell responses to HIV-1 antigen were absent in both patients. Ag/Abs to HIV-1 were detectable by CMIA and WB in both patients prior to BMT. Post-transplant, both patients had low level detectable Ag/Abs on CMIA, but by WB there was only trace antibody detectability in patient A and absent antibodies in patient B.

Conclusions: Assessment of the HIV-1 reservoir size in these two patients after allogeneic BMT with RIC shows undetectable HIV-1 RNA and DNA in peripheral blood and absent CD4+ T cell responses to HIV-1 antigen. We also found a profound reduction in HIV-1 Ab detectability in both patients by WB.
**Immunological Correlates of HIV-1 DNA Decline During Latency Reversal with Panobinostat in Patients on Suppressive cART**

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**Background:** Reversing HIV-1 latency is a possible strategy to reduce the HIV-1 reservoir, but elimination of latently infected cells likely depends on immune mechanisms. In a clinical trial that included 15 HIV-patients, 8 weeks of cyclic therapy with the HDACi panobinostat, significantly increased HIV-1 transcription and plasma HIV-1 RNA. At cohort level, HIV-1 DNA did not change but persistent reductions in total HIV-1 DNA were observed in some patients. Here, we report associations between changes in HIV-1 DNA during panobinostat treatment and 1) viral rebound during analytical treatment interruption (ATI); and 2) immune effector characteristics.

**Methods:** During ATI, viral load and CD4 T cell counts were monitored twice weekly. Total and integrated HIV-1 DNA in CD4 T cells was determined using digital droplet PCR. HIV-1-specific CD8 T cells were analyzed using interferon-γ elispots with an individual HLA-class I-matched library of epitopic peptides. Frequencies and phenotypic characteristics of NK cells, T cells, as well as regulatory T cells were determined before, during, and after panobinostat treatment using multiparametric flow cytometry.

**Results:** Viral rebound occurred in all ATI participants (n=9) with a median time to viral rebound >1,000 copies/ml of 17 days (range 14-56). Time to viral rebound correlated significantly with reductions in both total (p=0.0003) and integrated HIV-1 DNA (p=0.018) during panobinostat treatment. The breadth and magnitude of HIV-1-specific CD8 T cell responses to HIV peptides expanded significantly during panobinostat treatment, but this was not correlated to changes in HIV-1 DNA. However, low baseline levels of PD-1 expressing CD8 T cells strongly predicted a sustained decrease in HIV-1 DNA levels (p=0.001). Further, declining HIV-1 DNA levels during panobinostat treatment were associated with increasing proportions of activated (CD69+) NK cells (p=0.04) and decreasing expression of the inhibitory NK cell marker NKG2A (p=0.001). Levels of PD-1 on CD4 T cells as well as expression of inhibitory molecules CTLA-4 and CD39 on regulatory T cells were not associated with HIV-1 DNA changes.

**Conclusions:** In this exploratory analysis, we identified key innate and adaptive immune effector characteristics that correlated with HIV-1 DNA changes during latency reversal. Combining panobinostat with immunotherapy may result in improved elimination of the HIV-1 reservoir.
**PE50**

**Agnostic antibody to human IL-21 promotes cytotoxic immune response to control viral infection**

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**Background:** Combination antiretroviral therapy (cART) does not eliminate Human Immunodeficiency Virus (HIV) due to the persistence of latently infected cells. One strategy of eliminating latently infected cells is to reactivate the latent reservoir of virus in infected cells, which might result in cell death via virus-induced cytolysis or immune clearance. Recent evidence from an in vitro model showed that viral activation-induced cytolysis was not sufficient and functional HIV-specific cytotoxic T-cell killing is required for the success of this strategy. Interleukin-21 (IL-21) has been shown to control the chronic infection via enhancement of cytotoxicity in CD8⁺ T cells and prevention of CD8⁺ T cell exhaustion in mouse models. In human chronic HIV infection, IL-21 also positively correlates with viral controls. IL-21 is therefore a promising candidate for the “shock and kill” approach to eliminate latently infected cells in HIV patients.

**Methods:** We have identified an agnostic monoclonal antibody (mAb) to human IL-21. The application of this agnostic mAb to IL-21 was tested in cultured peripheral blood mononuclear cells (PBMCs) from HIV-patients und cART for the cytotoxic immune response to HIV and in mice chronically infected with lymphocytic choriomeningitis (LCMV) for the cytotoxic immune response to control viral infection in vivo. The mechanism of this agnostic mAb was studied by crystallography.

**Results:** The agnostic mAb strongly enhanced IL-21 function to promote the cytotoxicity of CD8⁺ T cells and NK cells ex vivo. The function of agnostic mAb in vivo is being evaluated. Structural study reveals the agnostic mAb binds approximately to the IL-21-IL-21 receptor interaction interface and can stabilise a more active conformation to enhance IL-21 bioactivity.

**Conclusions:** IL-21 is a suitable candidate to promote cytotoxic immune responses for the “shock and kill” approach to eliminate latently infected cells in HIV patients. The agnostic mAb to human IL-21 has a promising therapeutic potential. Its future development is warranted.
Mixed Methods, Integrated Approaches and Synergies in HIV Research and Intervention

PE51 Stakeholder engagement in HIV cure research - Lessons learned from other interventions and the way forward

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Background: Clinical and basic science advances have raised considerable hope for achieving an HIV cure, accelerating research in a number of related fields. The research is dominated primarily by concerns regarding the conduct and progress of the current trials, the nature and design of future trials and the preparedness of potential patients at the individual level. Given the complexity of curing diseases generally, and HIV in particular, a comprehensive social and policy analysis is needed to accompany clinical cure research. Addressing the role of stakeholders in the socio-cultural context of HIV cure may provide valuable information to guide the implementation of research and future programs.

Methods: We review stakeholder engagement in previous HIV research and implications for future HIV cure research. A research agenda to examine the engagement and preparedness of stakeholders in this long-term process is proposed.

Results: The experience from HIV vaccine development shows that early engagement of stakeholders helped managing expectations and mutual learning curves, mitigating the fallout of failure of several efficacy trials, and paving the way to continuum of clinical research. On the other hand, the relatively late engagement of HIV stakeholders in pre-exposure prophylaxis and adult medical male circumcision research may partly explain some of the implementation challenges. Stakeholder engagement represents therefore a key investment where researchers and stakeholders mutually accept to share knowledge, benefits and risk of failure.

Conclusions /Next steps: As HIV cure research advances from early trials involving subjects with generally favorable prognosis to more complex research phases involving toxic drugs and treatment interruptions, success will require early and deliberate engagement of stakeholders. This strategy may facilitate the implementation and sustainability of future programs.
Community Engagement in Research and Research Dissemination

PE52 Global investment in HIV cure research and development in 2013: promising science and international collaborations

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Background: Since 2004, the HIV Vaccines and Microbicides Resource Tracking Working Group has employed a comprehensive methodology to track trends in research and development (R&D) investments and expenditures for biomedical HIV prevention options. In 2011, the Working Group expanded its data collection to include HIV cure research. In 2013, the Working Group collaborated with the International AIDS Society, amfAR and the Treatment Action Group to undertake a comprehensive data collection and analysis of investment in HIV cure research.

Methods: R&D data were collected on annual disbursements by public, private and philanthropic funders for product development, clinical trials and trial preparation, community education and policy advocacy in order to estimate annual investment in HIV cure R&D. The Working Group’s estimate of HIV cure research investment is based on the definition of cure research developed by the US National Institutes of Health’s Office of AIDS Research. Investment trends were assessed and compared by year, funder category and geographic location.

Results: Investment in research towards an HIV cure markedly increased between 2012 and 2013 following promising scientific developments. In 2013, the majority of funding for research towards an HIV cure came from the public sector, with research agencies in the US, Europe and Australia providing the majority of investment, and a limited amount coming from the philanthropic and private sector.

Conclusions: Investments made towards HIV cure research highlight international collaborations underway that have brought about scientific progress seen to date. The Collaborative HIV Eradication of viral Reservoirs, the amfAR Research Consortium on HIV Eradication, the IAS/ANRS Young Investigator Award Program and the Martin Delaney Collaboratories are a few of the cross-institutional collaborations moving the field forward in a period of increasing global fiscal austerity. The scientific progress from these collaborations allowed the field to further prioritize research towards an HIV cure. In 2013, the US National Institutes of Health announced US$100 million in AIDS research funding redirected to support HIV cure research over three years. With increased investment, it is imperative to continue to track resources and ensure international collaborations continue so resources are put to the best use and the field continues to move forward.