Session 1: In vitro and in vivo models of HIV persistence

PP 1.0
Characterization of humanized NSG mice to evaluate latency
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Background: A current challenge to efforts to eliminate HIV-1 from infected individuals is the establishment of persistent long-lived HIV infected cells in blood and various anatomic compartments. The resting memory CD4+ T cells are the best characterized HIV-1 reservoir and they can be detected in blood, lymph node and gut.

Methods: We have utilized NOD/SCID/γc null (NSG) mice for our studies as NSG newborn mice can be successfully reconstituted with human lymphoid and myeloerythroid components following fetal-liver derived HSC injection. Our results demonstrate that humanized NSG mice support production of human cell types permissive to HIV-1 infection. We also have mice to sustain long-term infection by infecting them with R5-tropic HIV-1 and viral infection was assessed by qRT-PCR and CD4+ T cell levels in peripheral blood were quantified by flow cytometry.

Results: Our results show that R5-tropic virus is capable of infecting humanized NSG mice as demonstrated by high levels of plasma viremia and that HIV-1 infection leads to CD4+ T cell depletion in peripheral blood, thus mimicking the key aspects of HIV-1 pathogenesis. The NSG mice with demonstrable HIV infection were treated for 6–10 weeks with combinatorial antiretroviral therapy composed of drugs that block new infections, but not drugs that inhibit the viral production of infected cells.

Conclusions: The treatment blocked emergence of viral RNA, as expected and plasma viremia was confirmed to be below detectable limits within 4 weeks following initiation of treatment in all animals. The persistence of HIV during antiretroviral treatment is due to the latent reservoir. Graft-versus-host (GVH) responses likely result in clearance of residual recipient cells harboring HIV. Beneficial GVH responses, which permit donor cells to clear tumor or residual host hematopoietic cells, may play an important role in surveillance and clearance of residual HIV-infected cells following HSCT.

PP 1.1
Ex vivo determination of stem cell transplantation graft-versus-HIV reservoir effects
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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is one of the few strategies that substantially reduces HIV-1 reservoir size. Graft-versus-host (GVH) responses likely result in clearance of residual recipient cells harboring HIV. Beneficial GVH responses, which permit donor cells to clear tumor or residual host hematopoietic cells, may be mediated largely by the innate immune system. To investigate the role of NK cells and other lymphocytes in reactivating and eliminating latent HIV following HSCT, we designed a novel ex vivo assay to determine the activity of HLA-matched, post-HSCT donor effector cells on latently infected, pre-HSCT host CD4 T cells.

Methods: We adapted a latency model to enable infection of high numbers of CD4 T cells from individuals with hematopoietic malignancies prior to HSCT with an iGFP-gag HIV viral strain. The infected pre-HSCT CD4 T cells were then co-incubated with PBMC obtained from the same individuals 9–12 months after HSCT, and following full donor cell chimerism. We then determined lymphocyte activation, proliferation, viral reactivation and death over a 2 week period using flow cytometric analyses.

Results: We included samples from a total of 30 HIV-negative individuals who received either full myeloablative or reduced intensity HSCT. Up to 95% pre-HSCT CD4 T cells were infected with iGFP-HIV-1, with subsequent resulting in large numbers of latently infected cells. Flow cytometry was performed 0–13 days following lymphocyte mixing and co-culture. Of note, higher levels of non-proliferating HIV reactivated cells were found in the autologous setting compared to that of the allogeneic samples. Conversely, higher levels of proliferating HIV-infected cells were seen in the allogeneic samples, peaking at day 7. While expression of activation markers increased on NK, NKT and CD8 T cells, there were no differences found between the autologous and allogeneic groups. However, CD8 T cell activation was strongly correlated with HIV production (R2=0.975).

Conclusions: Our findings suggest that lymphocytes, including NK and NKT cells, may play an important role in surveillance and clearance of residual HIV-infected cells following HSCT.

PP 1.2
NNRTIs reduce HIV-1 production from latently infected resting CD4+ T cells
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Background: Clinical trials are investigating the potential for LRAs to reduce the size of the latent HIV-1 reservoir. In theory, LRAs “kick” HIV-1 out of latency which promotes the “kill” of the infected cell by viral cytopathic effects and/or the host’s immune response. Importantly, this approach is always carried out with ART to prevent de novo infection by the LRA-induced HIV-1. Here, we assessed whether different ARVs impact the “kick and kill” strategy.

Methods: Latency reversal was evaluated in a primary cell model of latency. Two hrs prior to addition of anti-CD3/CD28 antibodies, cells were treated with a PI (atazanavir, darunavir), NRTI (lamivudine), NNRTI (rilpivirine, efavirenz) or INSTI (raltegravir). Controls included cells that were exposed to antibody only or to ARVs only. Seven days post antibody administration cell-associated DNA and extracellular virion-associated HIV-1 were quantified. T cell activation and viability were assessed by flow cytometry.

Results: NNRTIs were found to decrease HIV-1 production (by ~2-log fold-change) in resting CD4+ T cells exposed to anti-CD3/CD28 antibodies. This decrease in HIV-1 production was not due to toxicity, or the NNRTI impacting CD25, CD69 or HLA-DR expression in the absence or presence of anti-CD3/CD28 antibody. In contrast, none of the other ARVs, including PIs which target the late stages of HIV-1 replication, had a significant impact on virus production. Despite the decrease in virus production, HIV-1 DNA declined by ~85% in the NNRTI-treated cells, which suggests decay of the latent reservoir. We hypothesize that the NNRTIs enhance premature activation of HIV-1 PR that results in intracellular processing of Gag and Gag-Pol, decreased viral particle production, and cellular toxicity.

Conclusions: NNRTIs reduce HIV-1 production from latently infected cells. Ex vivo studies that use NNRTIs to prevent virus spread or cells from donors on NNRTI containing regimens should be cautiously interpreted.
Towards achieving a state of reversible HIV-1 latency in primary monocyte-derived macrophages (MDM) by M1 polarization

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Background: Whether myeloid cells are latently infected remains to be firmly established. In this regard, we have previously reported that short-term exposure of primary MDM to pro-inflammatory cytokines (IFN-γ plus TNF-α), i.e. “M1 polarization”, partially prevented productive virus infection and reduced proviral transcription.

Methods: M1-polarized MDM were restimulated with M1 cytokines (IL-12, IFN-γ, TNF-α). Cell cultures were monitored for supernatant-associated RT activity, HIV-1 DNA load, APOBEC3G/3A expression, and for cell reactivation by co-culture with T cell blasts.

Results: We observed a significant, further reduction of virus replication down to near undetectable levels by RT activity over 30 days of culture. HIV-1 DNA levels were ca. 100- and 1,000-fold lower in M1-MDM than in control, infected MDM. No effect of T cell blast co-culture on control, infected MDM was observed, whereas significant levels of RT activity were induced in M1-MDM by this approach.

Conclusions: Stimulation of already infected M1-MDM with pro-inflammatory, M1-cytokines counterintuitively resulted in a further, significant inhibition of virus replication down to “near-latency” levels in terms of RT activity and viral DNA levels and upregulation of APOBEC3A expression. Recovery of virus production was achieved by cocultivation of M1-activated MDM with allogeneic T cell blasts, indicating the existence of a pool of infected cells carrying inducible proviruses.

Co-culture of T-cells with dendritic cells facilitates HIV latency in proliferating CD4+ T-cells: implications for the establishment and reversal of latency

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The major barrier to HIV cure is latently infected resting CD4+ T-cells. Previously we showed that myeloid dendritic cells (mDC) and monocytes induce latency in non-proliferating CD4+ T-cells, and we now examined whether latency is established in proliferating CD4+ T-cells under similar conditions. Proliferation dye labeled resting CD4+ T-cells were cultured alone or with syngeneic mDC, plasmacytoid DC (pDC) or monocytes in the presence of staphylococcal enterotoxin B (SEB). After 24h, cultures were infected with CCR5-tropic enhanced green fluorescent protein (EGFP)-reporter HIV. Five days post-infection, non-producection-infected, non-proliferating and proliferating T-cells were sorted and cultured until day 12 with IL-7, fusion inhibitor and integrase inhibitor. On day 5 and 12 post-infection, EGFP was quantified after activation with αCD3/αCD28/IgBL as a marker of latency. Latency was detected in non-productively infected, proliferating T-cells co-cultured with mDC, pDC and monocytes (median(IQR), 4(0.5–15), 0.4(0.01–2), 1.7(0.01–10); n=15). Using ALU-LTR integrated HIV DNA was only detected in mDC and monocyte co-cultures (10% each; n=17). At day 12, latency was detected in proliferating T-cells co-cultured with mDC and monocyte, but not pDC where cell viability was below 20% (n=4). At day 5, proliferating CD4+ T-cells from mDC, pDC and monocyte co-cultures highly expressed CD25 (97, 86, 99% respectively; n=6), C699 (39, 32, 46%; n=5), Tim-3 (23, 67, 48%; n=4), and had low expression of PD-1 (0.1, 1.9, 3%; n=4) and K67 (7, 18, 11%; n=7). At day 5, measurement of T-cell receptor Vβ chains that were SEB specific (17, 3) and non-specific (13.1) showed no enrichment on non-proliferating or proliferating T-cells, suggesting that latency establishment is independent of cognate interaction. Our data shows that latently infected proliferating cells may be an important mechanism for the establishment of latency and should be included in future studies of HIV persistence.
Background: The estrogen receptor type 1 (ESR-1) recruits specific repressive complexes to the HIV LTR. This study was designed to test whether ESR-1 modulates gender specific differences in the control of HIV latency.

Methods: ESR-1 was identified an essential factor used to maintain HIV latency by unbiased shRNA library screens. Specific agonists and antagonists of ESR-1 were used to inhibit or promote HIV reactivation. Patient cells from 10 well-matched male and female donors were evaluated by a novel assay for induction of spliced env-mRNA based on next-generation sequencing (EDITS assay).

Results: Antagonists of ESR-1 (tamoxifen and fulvestrant) are weak proviral activators but sensitize latently infected cells to low doses of TNF-α (NF-κB inducer) and SAHA (HDAC). Blocking of co-activator 3 (SRC-3), an upstream modulator of ESR-1, by gossypol also induces latent proviruses. By contrast, ESR-1 agonists, (propylpyrazoletriol, diethylstilbestrol, β-estradiol) strongly suppress both TNF-α and SAHA reactivation of latent proviruses. Chromatin immunoprecipitation (ChiP) assays show ESR-1 accumulates on the latent proviral genome. In HAART-treated patient samples there was a small increase of spliced HIV env mRNA when resting memory cells were treated with fulvestrant or tamoxifen. Proval reactivation by these ESR antagonists was synergistically increased by SAHA and IL-15. β-estradiol at concentrations in the physiological range led to dramatic reductions in proviral reactivation. Importantly, females showed higher levels of inhibition in response to β-estradiol and higher reactivity in response to ESR-1 modulators than males.

Conclusions: ESR-1 is a pharmacologically attractive target that can be exploited in the design of therapeutic strategies aimed at eradication of the latent reservoir. Clinically useful drugs targeting ESR-1 can be used to either promote the re-activation of latent proviruses (agonists) or limit their responses (agonists). The profound effects of β-estradiol on HIV reservoir reactivation suggests there may be gender specific differences in HIV reservoirs and highlights the need to tailor latency reactivation strategies for both men and women.

PP 2.1

HIV-1 silencing mediated by TRIM22 inhibition of Sp1 binding to the promoter

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Background: We have previously described that IFN-inducible TRIM22 is a suppressor of basal and PMA-dependent transcription acting independently of NF-κB and Tat/TAR. We have here investigated whether TRIM22 could interfere with such Sp1-driven transcriptional activation of HIV-1 LTR.

Methods: 293T cells, lacking of endogenous TRIM22, were co-transfected with a TRIM22-expressing plasmid together with reporter vectors driven by the HIV-1 promoter containing either wild-type or mutated Sp1 binding sites or lacking of either one or two sites; reporter expression was assessed 48 hours post-transfection. Endogenous TRIM22 was knocked-down (KD) in SupT1 cells that were subsequently infected with HIV-1 molecular clones engineered to be dependent on an incorporated Tet-On gene expression system for activation of transcription while being independent of Tat/TAR interaction. Virus replication was monitored up to 32 days post-infection. Cell extracts from TRIM22-transfected 293T were subjected to i) immunoprecipitation, ii) Western blotting iii) DNA pull-down and iv) Chromatin Immunoprecipitation (ChiP).

Results: TRIM22 overexpression suppressed Sp1-driven transcription of HIV-1. As its inhibitory activity was lost in the absence of Sp1 binding sites. In contrast, TRIM22 KD increased the replication of infectious clones that were exclusively dependent upon Sp1 binding to the promoter. Furthermore, immunoprecipitation experiments showed that TRIM22 did not interact with Sp1 and did not directly bind to the HIV-1 LTR, however TRIM22 expression drastically prevented Sp1 binding to the HIV-1 LTR.

Conclusions: TRIM22 inhibits Sp1-dependent transcription by preventing Sp1 binding to the HIV-1 LTR likely through the recruitment of factors that remain to be defined. Our findings bear relevance for the discovery of novel factors that mediate HIV-1 transcriptional silencing.

PP 2.2

Viral counteractions against CTIP2 in HIV-1 permissive cells

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Background: Latently infected cells constitute major blocks to an HIV-1 eradication and a functional cure of the patients. We have previously reported that the cellular co-factor CTIP2 plays a key role in the establishment and persistence of HIV latency in microglial cells, the main reservoirs of the virus in the brain. By recruiting large enzymatic complexes at the viral promoter, CTIP2 silences HIV-1 gene transcription and disfavors the viral reactivation from the reservoirs. However, nothing is known on how can HIV-1 counteracts the effects of CTIP2 in permissively infected cells. Unsealing the host ubiquitination machinery to target undesirable host proteins is a common strategy utilized by retroviruses. Here, we tend to postulate that HIV-1 Vpr may target CTIP2 by Cui4A-DDB1-DCAF1 complex to counteract its effects on HIV-1 replication.

Methods: Investigations were performed at the biochemical, molecular and cellular levels.

Results: We demonstrated that interferon treatments induce expression of CTIP2 at the mRNA and the protein levels suggesting that this factor may be part of the cellular response to viral infections. We observed that replication of WT- but not Vpr-deleted HIV-1 reduced CTIP2 expression in productively infected cells. Vpr expression was correlated with low levels of CTIP2 and increased levels HIV-1 gene transcription. In addition, co-immunoprecipitation experiments showed that CTIP2 interacts with DDB1, DCAF1 and HIV-1 Vpr in order to induce the degradation of CTIP2 by proteasome. Finally, the abrogation of Vpr binding to the DCAF1-CUI4-DDB1 complex prevented CTIP2 degradation.

Conclusions: Our results suggest that Vpr engages the ubiquitination machinery to induce CTIP2 degradation. By degrading CTIP2, HIV-1 counters CTIP2-mediated silencing of its expression and thus favors viral replication.

PP 2.3

The unique enrichment of histone modifications and its relationship with HIV-1 latency in some chromosomes

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Background: Histone modifications such as acetylation and methylation play a central epigenetic role in the organization of chromatin domains and the up/downregulation of gene expression. Although many studies have reported that the epigenetic mechanism is strongly involved in the maintenance of HIV-1 transcriptional latency, the epigenetic control of viral replication and how HIV-1 latency maintains are not fully understood.

Methods: We used the high-throughput parallel DNA sequencing (ChiP-seq) approach to investigate the effect of histone modifications, H3K4me3 and H3K9ac, on HIV-1 latency. ChiP-seq outputs from
CD4+ T cell line (A3.01) and HIV-1 latently infected cells (ACH2, J1.1, and NCHA1) were aligned to hg18 using bowtie and analyzed with SICER, CEAS, HOMER, Webgestalt, and PCVis.

**Results:** Chromosomes 16, 17, 19 and 22 were significantly enriched for histone modifications in both decreased and increased islands. 38 decreased islands from 126 H3K4me3 islands and 302 H3K9ac islands common at specific chromosomes in HIV-1 latent cells and 41 increased islands from 130 H3K4me3 islands and 164 H3K9ac islands were selected for functional annotation. In Gene Ontology analysis, 38 genes were involved in regulation of biological process, regulation of cellular process, biological regulation and purinergic receptor signaling pathway. 41 genes were in nucleic acid binding, calcium activated cation channel activity, DNA binding and zinc ion binding. By pathway commons analysis in Webgestalt, 38 genes were strongly involved in p63 transcription factor network and 41 genes were in RNA polymerase III transcription termination pathway. TOP2A, ITGB4, TRAF4, SEC14L2, NFIC and NFXW were selected as candidates for HIV latency. Additionally, TRAF4 was found to directly interact with NFXW.

**Conclusions:** The unique enrichment of histone modifications and its cross-talk in specific chromosomes might play a crucial role in the establishment and maintenance of HIV-1 latency.

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**PP 2.4**

**Exosomes from HIV-1 infected cells stimulate production of pro-inflammatory cytokines through TAR RNA.**

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**Methods:** Exosomes were isolated from culture supernatants and sera using differential ultracentrifugation or by nanotrap particle capture. The levels of the TAR RNA were quantified from purified exosomes by RT-qPCR.

**Results:** TAR RNA was quantified in the exosomes of infected cells. Levels of the TAR RNA were increased in the presence of the exosomes.

**Conclusions:** Exosomes from HIV-1 infected cells stimulate production of pro-inflammatory cytokines through TAR RNA.

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**PP 2.5**

**Ingenol derivatives are potent reactivators of latent HIV**

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**Methods:** HIV latency was modeled in primary T cells using a replicon competent virus (HIV-1KQN). Ingenol and ingenol derivatives (10 nM, 1 μM, 100 nM, and 10 nM) were tested for their abilities to reactivate latent HIV. Additionally, ingenol derivatives were tested in cells from aviremic patients via the Rapid Ex Vivo Evaluation of Anti-Latency (REVEAL) assay, in which virion-bound HIV-1 genomic RNA was quantified.

**Results:** Ingenol derivatives successfully reactivated latent HIV at comparable levels to αCD3/αCD28. Optimal reactivation and viability was seen with the addition of a 100 nM concentration of ingenol derivatives. The ingenol core itself was devoid of activity. Additionally, ingenol 3,20-dibenzoate increased viral mRNA copies to a greater extent than panobinostat and other HDAC inhibitors ex vivo. All ingenol derivatives were found to induce release of select cytokines in a donor-dependent fashion, and to lack cytotoxicity in primary cells.

**Conclusions:** Ingenol derivatives are promising LRAs and our lab is pursuing novel methods to chemically engineer a library of ingenol derivatives with combinatorial substitutions modifying activity and specificity.

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**PP 2.6**

**LEDGF/p75 and Iws1 participate both cooperatively and independently to distinct steps of HIV transcription**

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**Methods:** Ingenol derivatives are potent reactivators of latent HIV-1. We have recently identified a novel complex involved in HIV latency that contains LEDGF/p75 together with transcription factors LHS1 and Sp6. Using biochemical approaches, we characterized the interaction interfaces between LEDGF/p75 and LHS1. We showed that in activated CD4+ T cells, both LEDGF/p75 and LHS1 contributed to the maintenance of HIV latency. ChIP experiments revealed that LEDGF/p75 and LHS1 colocalized with RNAPII at the viral promoter in latently infected cells. Upon PMA stimulation of HIV transcription, LHS1 and RNAPII levels strongly increased within the HIV genome, whereas LEDGF/p75 distribution was not affected. In addition to HIV, we found that LEDGF/p75 and LHS1 are also recruited at the cellular c/Myc gene in Jurkat cells. LEDGF/p75 accumulates within the gene body.
of c-Myc with a pattern similar to that observed for lws1 and H3K36me3. Thus, our results suggest that LEDGF/p75 and lws1 both cooperatively and independently to distinct steps of chromatin transcription regulation.

PP 2.7

Constraints on the dynamics of HIV-1 lifecycle elucidated by treatment with an integrase inhibitor reveal a subset of cells with very slow integration

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Background: Mathematical models of HIV antiretroviral therapy have shed light into the biology of the virus. We compared viral kinetics under treatment with and without integrase inhibitors to gain new insights into this step of the lifecycle, which is crucial in defining latency and the potential to avoid new rounds of replication under shock-and-kill.

Methods: We developed a model of HIV infection explicitly accounting for proviral integration to analyze therapy containing integrase inhibitors. We validated the model with data from 28 HIV-1 infected participants treated with raltegravir (RAL) monotherapy for 9 days and compared these dynamics with data from nine HIV-1-infected individuals treated with a highly active quad-regimen including raltegravir-ritonavir, efavirenz, lamivudine, and tenofovir DF. Plasma HIV-1 RNA was measured frequently and we fitted the data using mixed-effect models.

Results: The model predicts two phases of viral decay early on (phase 1a and 1b), as we have shown recently for combination therapy (RAL and reverse transcriptase inhibitors). Phase 1a corresponds to loss of productively infected cells (half-life of ~19h) and phase 1b reflects the loss of infected cells with non-integrated provirus. When we analyzed the decay with the quad regimen, we also found a two-phase decay early on. However, here the slope of the second phase was slower than phase 1b with RAL. The model predicts that this second phase slope is due to loss of a small subset of cells with very slow integration, up to 100-fold slower than in activated short-lived infected cells.

Conclusions: Analyses of HIV viral dynamics under treatment allows us to analyze possible sources of infected cells. We speculate that the cells with very slow integration could be resting cells, which are a potential source of latently infected cells. In any case, the time window for the immune system to kill these infected, but not productive cells, is much longer than previously thought.

PP 2.8

The steroid receptor coactivators are targets for reactivation of HIV latency

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Antiretroviral therapy (ART) is effective for restricting the onset of AIDS in HIV-infected individuals. Unfortunately, withdrawal results in viral rebound and HIV patients must maintain life-long therapy of the drug cocktail. Recent evidence suggests potential for pharmacological eradication of HIV, yet proviral transcriptional events involving host chromatin present a challenge that is relatively understudied. Modulation of the latter has therapeutic implications for controlled induction of proviral expression in latent cell populations resistant to ART, with potential for “shock-and-kill” eradication. Here, we reveal pharmacological targeting of the steroid receptor coactivator (SRC) family of transcriptional coactivators can potently activate HIV, and SRC-3 is an obligate host factor for efficient proviral transcription. A screen designed to alter SRC-3 intrinsic activity using nearly 360,000 compounds identified several pharmacological agents capable of inducing LTR-reporter based transcription. To confirm these results in a T-cell model of HIV latency, we employed the 2D10/d2GFP Jurkat latency (J-Lat) model to test proviral activation upon treatment with compounds targeting SRC-3. Two SRC-3 activating compounds double the number of GFP positive cells at micromolar concentrations, while lead molecule MCB613 displays at least 5-fold activation in the nanomolar (nM) range. The SRC-3 activating compound MCB613 is synergistic with known HIV activating compounds SAHA and JD1 in 2D10 cells. MCB613 directly binds to SRC-3 and promotes secondary recruitment of histone acetyltransferases CBP and p300 in vitro. Moreover, chemical derivatives of MCB613 activate HIV d2GFP reporter activity at low nM ranges and have no toxicity in primary T cells and PBMCs. Collectively, these results underscore the importance of host coregulators in HIV transcription and identify novel targets for preclinical studies seeking to activate latent HIV reservoirs.

Session 3: Clinical virology of HIV persistence

PP 3.0

Allogeneic stem cell transplantation in HIV-1 infected individuals

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To date, the only and most compelling evidence of a medical intervention that has been able to cure HIV-1 infection (the “Berlin patient”), involved an allogeneic stem cell transplant (SCT) from a donor who was homozygous for CCR5Δ32. Although this high-risk procedure is only indicated for certain hematological malignancies, the strategy raised tremendous scientific potential to fully understand the bases of such success. Unfortunately no other successful long-term outcome of allogeneic SCT has been published since the Berlin patient. This may at least partly be explained by the relatively rare frequency of the homozygous CCR5Δ32 genotype coupled with the need for stringent HLA matching. Alternative strategies that allow for less stringent matching such as double cord transplantation, or single cord blood transplantation followed by the co-infusion of CD34+ cells from a third party HLA-mismatched donor may increase the chances of selecting a CCR5Δ32 donor.

Detailed analysis of these cases should provide insight as to whether additional factors such as conditioning regimen, total body irradiation and graft versus host disease contributed to the eradication of the potentially infectious viral reservoir in addition to the lack of a functional CCR5 receptor.

During the last year EpiStem consortium has generated a prospective observational cohort of 15 cases of allogeneic SCT in HIV-1-infected individuals with life-threatening hematological malignancies around Europe. Five cases had a CCR5Δ32 donor and 10 had a CCR5Δ32w donor. In three cases the donor cells came from a cord blood unit and in 12 cases from an adult donor. So far three patients have successfully passed the 6 months follow-up after transplantation, and six patients have died after transplantation, despite achieving full donor cell chimerism in some cases. Preliminary analysis of virological and immunological data from blood and tissue samples shows a systematic reduction of HIV-1 reservoirs to very low levels. Moreover, cord blood units in multiple European blood banks and adult donors have been genotyped for CCR5 to generate a registry of CCR5Δ32 available donors.
PP 3.1
Genetic and functional characterization of HIV-1 Nef gene from North Indian HIV-1 infected patients
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Background: Designing an ideal vaccine against HIV-1 has been difficult due to enormous genetic variability as a result of high replication rate and lack of proof reading activity of reverse transcriptase leading to emergence of genetic variants and recombinants. Genetic studies reveal that HIV-1 Nef gene shows extensive genetic diversity. Functional studies have been carried out mostly with Nef derived from subtype B pNL4-3 virus. The rationale of this study was to characterize genetic variations that are present in the nef gene from HIV-1 infected individuals from North-India and determine their functional implications.

Methods: Genomic DNA was isolated from PBMCs of HIV-1 infected patients and nef gene was PCR amplified with specific primers followed by cloning, sequencing and sequence analyses using bioinformatics tools for predicting HIV-1 subtypes, recombination events and conservation of domains. The unique representative variants were then characterized with respect to their ability to downregulate CD4 and MHCI-1 expressed on cell surface.

Results: Phylogenetic analysis of nef variants revealed sequence similarity with consensus subtype B and B/C recombinants. Bootscan analysis of some of our variants showed homology to B/C recombinant and some to wild type nef B. High amino acid variations was observed among our most of the variants. dN/dS ratio revealed 80% purifying selection and 20% diversifying selection implying the importance of variable mutations of Nef variants. There were some variants that possessed mutations in the functional domains of Nef responsible for its CD4 and MHC-I activity.

Conclusions: We observed enhanced biological activities in some of our variants may be the result of amino acid substitutions in their functional domains. In summary, the CD4 and MHC-I downregulation activity of Nef must be used by virus to its maximum advantage.

PP 3.3
Mild cognitive impairment in a clinically latent HIV-1 patient population
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Background: Given increased longevity of patients with HIV infection, there is interest in the intersection of HIV and age-associated cognitive disorders. Mild cognitive impairment (MCI) is associated with increased dementia risk and is defined as cognitive decline greater than typical aging. We used modified Jak et al. (2009) criteria to identify the prevalence and neuropsychological (NP) profile of MCI among HIV-1-infected patients on antiretroviral therapy (ART).

Methods: 171 HIV-1-infected patients (89% black; 67% male) completed NP tests of verbal fluency, working memory, executive function, episodic memory, and psychomotor speed. Scores adjusted for age, race, gender, and education were subjected to principal component analysis (PCA).

Results: A 3-factor structure was obtained (66% of variance) consisting of motor (23%), memory (22%), and executive tests (21%). Three groups were constructed: 43 patients (25%) meeting criteria for MCI, defined as a score at least 1 SD below normative values on at least two PCA-derived scales; 52 (30%) who scored below cutoff on only one PCA-derived scale; and 76 (44%) who scored above cutoff on all scales. Within-group t-tests indicated that the MCI group demonstrated poorest performance on memory tests (P<0.001). The groups did not differ in age (52±8), education (12±2), years seropositive (19±8), ART duration (12±7), or CD4 count (553±365). However, the MCI group reported greater functional difficulties than the other groups (P<0.01).

Conclusions: Nearly a quarter of our sample met criteria for MCI. Patients with MCI did not differ from patients without MCI in demographics or CD4 count; however, they reported greater functional difficulties. Poor memory typified the level of HIV-1 in patients with MCI and increased risk for dementia. Longitudinal assessment is needed to determine this outcome.
Towards HIV reservoir measurements in ART-treated patients: integrated DNA quantification and HIV-1 clone expansion in a Japanese cohort
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Background: Despite successes of ART, persistence of HIV reservoir and its measurement remain unsolved issues. Understanding the integrated viral DNA and the mechanisms of persistence are important for the future HIV eradication. Therefore, our aim was to evaluate the integrated HIV proviral DNA/reservoir in PBMC, proviral load-PVL) in a cohort of Japanese patients on ART.

Methods: A retrospective study of PBMC samples collected from around 100 patients of Kumamoto University hospital in the period 1996–2015 was performed. Patients were subdivided into groups according to current ART and its total length. PBMC DNA samples were run in a qPCR and percentage of integrated proviral DNA was calculated. Ten patients with long-term stable ART were selected and integration sites analysis via LM-PCR and NGS (Illumina) was performed at two time points: early therapy and recently. Integration sites and expansion of the HIV-1 clone were traced under in vivo conditions of suppressive long-term ART.

Results: Levels of integrated proviral DNA were low (mean PVL for the 10 patients: 0.016%) and remained relatively stable over the years under ART and with similar trends when comparing patients receiving different ART protocols. Integration site analysis was performed with PBMC DNA samples at a recent time point vs. upon early ART initiation point and the result showed stable levels of clone expansion with similar trends among the ART groups.

Conclusions: Our data identifies some of the patterns of reservoir persistence in PBMC and its evolution under successful long-term ART. Despite suppressive ART the levels of integrated DNA remain stable and the expansion of the HIV-1 clone successfully continues. The latter might suggest more precise therapy, better clinical monitoring tools or both are needed for routine evaluation and management of the HIV reservoir.

Impact of a decade of sustained antiretroviral therapy started during HIV-1 seroconversion on blood and gut reservoirs
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Background: Early ART limits the viral reservoir size and enhances the possibility of a functional cure. We have assessed the impact of such a strategy over a decade of successful treatment on the HIV-1 DNA reservoir.

Methods: Cross-sectional study of HIV-1 reservoir size (total and integrated HIV-1 DNA) and dynamics (2-LTR circles and cell-associated HIV-1 unspliced RNA (ssRNA)) performed using digital PCR in PBMCs of 84 HIV-1 patients (four cohorts: long-term treated patients with ART initiated during seroconversion, SRCV on ART, n=25; or chronic infection, Chronic ART, n=32; long-term non-progressors, LTNP, n=17; and ART-naïve recent seroconverters, Recent SRCV, n=10). Time on ART, CD4 count and CD4/CD8 ratio were collected. Rectal biopsies were taken on one occasion.

Results: Median total HIV-1 DNA copies were: 92, 48, 137 and 1901 copies/mL on ART was recruited in November 2012 in the RWMC patient G. Nuñari, S. Migueles, U. O’Doherty
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chronic progressors (CP) and four patients on ART. Genomic DNA was extracted from PBMCs and integrated HIV DNA measured by Alu-HIV PCR. Cytotoxic responses were monitored over time in EC measuring infected CD4 T-cell elimination (ICE) and granzyme B activity. Differences between time points were tested using paired t-tests or Wilcoxon Signed-Rank.

Results: Integrated HIV DNA increased over time in EC, from 5.1 (IQR 1.83–17.5) to 16.9 (IQR 5.6–40.2) copies/million PBMCs after 4.13 years (P = 0.0017) and increased more dramatically in CP, from 73.5 (IQR 29.75–118) to 1570 (IQR 670–1971) copies/million PBMCs after 6.1 years (P = 0.0019), but not in patients on ART, 1179 (IQR 632–1287) vs. 1038 (IQR 555–1074)/million PBMCs after 4.5 years. The median annual fold-change was significantly higher in CP compared to EC (10.5 vs. 1.28 per year, P = 0.006). CTL function as assessed by ICE (72.3 ± 7.54%, P = 0.44) and granzyme B activity (43 ± 56%, P = 0.13) did not significantly change over time in EC.

Conclusions: In the absence of ART, integrated HIV integration accumulates over time both in CP and, more slowly, in EC despite robust CTL activity. Low, albeit continued reservoir accumulation in EC might be due to a ongoing, low level rounds of replication in immunologic sanctuaries where CTL are excluded. Meanwhile, the rate of accumulation is more brisk in chronic progressors who have poor CTL function. The increase in integrated HIV DNA over time further reinforces the importance of treating non-controllers early to limit reservoir size and raises the question of whether it is reasonable to treat controllers at a certain threshold.

**PP 3.8**

**SNPs within the HIV–1LTR associate with increased virus persistence**

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Background: HIV-1 is continuously mutating, even in well-controlled individuals on cART. SNPs in the LTR are of interest due to their potential impact on the regulation of viral gene transcription and replication. Studies have previously demonstrated that SNPs in particular transcription factor (TF) binding sites can significantly alter viral transcription and correlate with changes in disease severity. To understand the molecular evolution of HIV-1 in patients longitudinally prior to and after initiation of cART, the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort in Philadelphia, PA was used to identify SNPs correlated with clinical disease parameters.

Methods: PBMCs were isolated from patients for PCR, sequencing and bioinformatic analysis. Linear mixed model analysis was used to identify SNPs that correlated with disease parameters. Jupyter analysis predicted the TF binding surrounding LTR nucleotide position 108 and electrophoretic mobility shift assay (EMSA) determined the direct TF/DNA interactions in T cells.

Results: Numerous hotspots were identified throughout the LTR. Position 108 change from A to G, residing in a confirmed COUP/AP1 binding site, was highly correlated with increase in viral load and a decrease in CD4+ T cell counts, even in individuals on continuous cART. EMSA demonstrated differential TF/DNA binding. Oligonucleotides with an A at position 108 showed three distinct complexes and a G at position 108 showed four distinct complexes.

Not only was a new complex formed with position 108 but the overall complex formation was increased. Supershift analysis supports the presence of numerous TFs as predicted by the multiple TF binding sites predicted in this region by JASPER analysis.

Conclusions: Even on cART, changes in the prevalence of SNPs in the LTR can be correlated with alterations in clinical disease severity involving enhanced viral persistence that may be driven by increased levels of transcription.

**PP 3.9**

**Detection of replication competent HIV from latently infected CD4+ T cells**

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Background: Accurate measurement of the latent HIV reservoir is critical for the evaluation of novel eradication strategies. Drawbacks of the ‘standard’ viral outgrowth assay are the high cost and poor sensitivity due to low expression of CCR5 on most donor PBMCs. RIVER, a UK wide collaborative on eradication is the first phase II trial designed to test a combination of a prime-boost vaccination strategy followed by treatment with an HDAC inhibitor in order to reduce the size of the latent HIV reservoir. This study aimed to develop a reliable assay to quantify the replication competent latent HIV load in CD4+ T cells with a significant cost reduction and increased sensitivity for R5-tropic virus.

Methods: Resting CD4+ T cells were obtained from whole blood using density-gradient PBMC isolation followed by negative selection of CD4+ T cells and depletion of activated cells. The resulting resting CD4+ T cells were activated with PHA and allogeneic irradiated PBMCs and co-cultured with a clonal cell line expressing CD4, CXCR4 and CCR5. Viral production was detected by HIV-1 p24 ELISA.

Results: The new assay has significantly lowered costs from £3800 to £750 per assay and is more sensitive than co-culture with donor PBMCs. Replication competent virus was detected in 19/23 patients. Re-stimulation with PHA of negative cultures often resulted in viral outgrowth in additional cultures. However, in samples from patients without a detectable reservoir all cultures remained negative upon re-stimulation. The robustness of the assay was validated using eight samples from the same patient taken over a period of 4 months.

Conclusion: The cell line based outgrowth assay reduced both labour and cost and has an improved sensitivity compared to a donor PBMC based assay. Multiple rounds of PHA stimulation result in a larger proportion of the latently infected population being activated, indicating that the standard assay is only a measure of the minimal reservoir size.

**PP 3.10**

**Low viral reservoir treated patients (LoViReT): clinical predictors of low HIV-1 DNA**

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Introduction: Small sized reservoirs are predominantly found in HIV-1 controllers and individuals who received ART during primary HIV-1 infection. Herein, we established a cohort of ‘Low Viral Reservoir Treated’ patients (LoViReT) with a very low HIV-1 DNA levels in peripheral blood despite having initiated ART during chronic HIV-1 infection. We looked for clinical and immunological variables that could explain the low level proviral reservoir observed in these subjects.

Methods: We recruited 319 patients on cART for 3–8 years. HIV-1 proviral reservoir was analyzed in PBMCs with ddPCR using two different primer sets, gag and ltr. HIV-1 env trimers antibody titers were determined using flow cytometry. Statistical analysis was carried out.
out with non-parametric methods and Spearman’s correlation coefficient.

**Results:** Total HIV DNA was detected in 96.2% of the samples, with a median proviral DNA of 136 copies/10^6 cells (IQR: 63–301). Patients were stratified in two groups: above and below this median. We observed lower HIV-1 DNA in younger patients with shorter time from diagnosis and lower levels of zenith plasma viremia. Additionally, low reservoir was associated with higher nadir CD4 T cells and higher CD4/CD8 ratio. We also found that patients who initiated therapy with raltegravir harbored a significantly lower reservoir.

LoViReT patients represented 20 subjects (6.3%) with HIV-1 DNA<10^6 copies/10^6 cells, including 12 undetectable patients. These subjects were younger, had been diagnosed more recently, and had higher nadir CD4 T cells when compared with the rest of the patients. We also observed that LoViReT patients had smaller envelope trimmer-specific antibody titers than patients with high latent reservoir.

**Conclusion:** LoViReT patients harbor an extremely low HIV-1 proviral reservoir during suppressive cART despite not being involved in any HIV eradication-based strategy. A more conserved immune system seems to characterize this infrequent cohort.

**PP 3.11**

**Population genetic approaches to estimating the size of the HIV reservoir**

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**Introduction:** Understanding the genetic characteristics of HIV populations is essential to characterizing the size the replicating HIV population as combination antiretroviral therapy (cART) is initiated. The size of the replicating viral population, which represents the reservoir of infectious HIV, has been reported to be substantial prior to cART, but variation in size among patients has not been extensively investigated. We used next generation sequencing (NGS) to obtain more accurate estimates of replicating population sizes and to investigate range in sizes of replicating HIV reservoirs prior to cART.

**Methods:** Longitudinal plasma samples from five chronically infected antiretroviral naïve individuals were subjected to NGS using a new library construction and processing strategy to obtain sequences with the illumina platform of c. 500-nt within the HIV RT. Replicating population sizes were estimated using temporal and coalescent methods, and linkage disequilibrium measures calculated (DNASP).

**Results:** Plasma samples from antiretroviral naïve patients (n=5) with chronic HIV infection (duration of infection>1 year), median CD4=546 cells/μL, viral RNA=3.9 log10 copies/mL were subjected to NGS, >15,000 sequences (average of 1,277 sequences/time point) were obtained. Estimates of replicating population sizes were consistently >1E5, and a a 100 fold range (1.7E5–2.4E7) of infectious cells per individual was detected among patients studied.

Linkage analyses revealed only 3–6 pairs of alleles in linkage disequilibrium (Fisher exact P<0.0001) for each patient time point, indicating extensive recombination.

**Conclusions:** HIV populations are well mixed prior to cART and have a broad range of replicating population sizes among patients. With cART, the range in sizes of HIV reservoirs capable of replicating is expected to be similarly wide.

**PP 3.12**

**Cell-associated HIV-1 DNA and viral load evaluation in HIV-1 infected children before and after combination antiretroviral therapy initiation**

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**Background:** Viral load (pVL) and CD4+ T cells counts are the main and effective measurements for HIV-1 infection monitoring. However, these parameters do not manifest the behavior of the viral intracellular DNA forms (CA-HIV-1 DNA) in the HIV-1 reservoirs. The aim of this study was determine CA-HIV-1 DNA levels and study its association with pVL in HIV-1 infected children before and after combination antiretroviral therapy (cARV).

**Methods:** Twenty-one children infected with HIV by vertical transmission, followed up from 2008 to 2013, were studied. Samples were tested prior to cART (pre-cARV) and at 18 months (± 6 months) after cARV initiation (post-cARV) with lopinavir/ritonavir and abacavir, didanosine or lamivudine. CA-HIV-1 DNA levels were quantified by a semi-nested real time PCR with Taqman probes targeting LTR-gag region in PBMCs (LLOD 8 copies/10^6 PBMC). The pVL data was collected.

**Results:** The median pVL at pre-cARV and at post-cARV was 5.7 (IQR 5.2–6.1) and 1.7 (IQR 1.7–2.2) log10copies ARN/mL, respectively. The median CA-HIV-1 DNA levels at pre-cARV were 1204 (IQR 346.8–2182.0) copies/10^6 PBMC, while at post-cARV they were 164.2 (IQR 63.9–359.3) copies/10^6 PBMC. This showed an important reduction in the levels of both variables: 70% in pVL and 86% in CA-HIV-1 DNA levels. Correlation between CA-HIV-1 DNA and pVL at pre-cARV was not found, while at post-cARV a positive correlation was found (r=0.49, P<0.05). Of 17 children studied at post-cARV, viral suppression, defined as consecutive pVL<400 copies HIV-1 ARN/mL, was observed in 14 (78%), with a median CA-HIV-1 DNA levels of 110 (IQR 63.3–1026) copies/10^6 PBMC. The four children who did not have viral control, median CA-HIV-1 DNA levels were 308.4 (IQR 239.6–370.4) copies/10^6 PBMC.

**Conclusions:** Our results suggest independent behavior between pVL and CA-HIV-1 DNA levels before treatment initiation. However, after 1 year of cARV, a significant reduction of both CA-HIV-1 DNA and pVL was observed and also a proportional counts of both.

**PP 3.13**

**Post-mortem analysis of HIV-1 reservoir after allogeneic transplantation using stem cells**

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**Background:** In the Berlin patient, cure of HIV infection was observed following stem cell transplantation (SCT) with homozygous CCR5Δ32 donor cells. In contrast, in the Boston patients, transplanted with a regular donor, rebound of HIV was observed after treatment interruption despite loss of detectable HIV DNA in PBMCs. It is unclear from which reservoir HIV rebounded.

**Methods:** We investigated the impact of SCT on the size of the HIV-1 reservoir in EpStem patient #11. SCT for acute myeloid leukemia was performed using an HLA-matched unrelated CCR5Δ32 donor. The patient was re-transplanted with cells from a CCR5Δ32 heterozygous donor after graft failure at 10 weeks. Before SCT we performed: (1) phenotypic and ultradeep genotypic (gp120-V3) co-receptor analysis; (2) quantitative analysis of the HIV reservoir in different CD4+ T-cell subsets (CD4+ T helper, CD4+ T cell memory, CD4+ T cell exhaustion and CD4+ T cell death) and bone marrow using ddPCR; (3) quantitative viral outgrowth assay (qVOA); (4) single copy assay (SCA) on plasma. Post-SCT viral dynamics and post mortem viral reservoir analysis on tissue were performed using ddPCR.

**Results:** Patient #11 was on effective cART for 18 years and harboured a subtype B CR5Δ-tropic virus population (false positive rate, 33–49%). Before SCT, no viral RNA was detected in routine diagnostics while 2 copies/mL plasma were observed in SCA. qVOA showed presence of replication competent virus (1.6 IUPM). Proviral DNA was detected in PBMCs (295 copies/10^6 cells), bone marrow (80 copies/10^6 cells), and CD4+ T cells with stem cell-like properties.
(490 copies/10^6 cells), naive T cells (579 copies/10^6 cells) and memory T-cell subsets (T_CEM, T_HOM and T_EM, 2237, 2854, and 4687 copies/10^6 cells, respectively). Five weeks after the second SCT, at time of full chimerism, proviral DNA declined to undetectable levels (<1 copies/10^6 PBMCs). Unfortunately, the patient thereafter died with a pneumonitis. Post mortem analysis revealed that proviral DNA could be detected in lymph node tissue (40 copies/10^6 CD4 cells) but not in ileum. **Conclusion:** Within EpiStem, we show that after 18 years of effective CART HIV DNA can readily be detected in various T-cell subsets. In the neutropenic phase post-SCT, HIV DNA could no longer be detected in PBMCs nor in ileum. In contrast, viral DNA was still found in lymph node tissue indicating that this tissue may serve as a long-standing viral reservoir after SCT.

**PP 3.14**

HIV antibodies as markers of HIV systemic reservoir and replication activity

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**Background:** Sensitive antibody (Ab) measurements are used to determine HIV infection. These tests can be modified to identify individuals who are within the seroconversion period and recently infected. Using these assays, we have shown significant decreases in Ab measures due to viral suppression or control. Longitudinal specimens from treated individuals can demonstrate Ab seroreversion due to reduction of circulating virus and viral reservoirs. We hypothesize that the concentration of Abs correlates with viral load and viral reservoir.

**Methods:** Modified HIV Ab assays were used to measure quantity and avidity of HIV Ab: less-sensitive (LS: dilution in buffer; increased cutoff) and avidity modifications (AI) VITROS Anti-HIV1 ET and 35 late-treated (LT) infected individuals at five timepoints before and after treatment. We measured changes of Ab over time, viral load measurements, and cell-associated RNA (CA-R) and proviral DNA (PV-D) measurements at the final timepoints.

**Results:** Ab from pre-treatment timepoints from chronically infected individuals correlate with viral load (LS- and AI VITROS P<0.005; LA< P<0.05). The seroreversion in all Ab is faster in the LT compared to the ET group (P<0.001) and for the LT, Ab concentrations continue to decline with viral suppression. The PV-D and CA-R measurements correlate with LA and LS-VITROS (both P<0.01). These differences were not observed in the ET or LT subgroup analysis.

**Conclusions:** There is a reduction in HIV Ab concentration and avidity with time on treatment and this correlates with pre-ART viral copies and post-ART viral reservoir. Measuring the quantity and quality of HIV Ab are a useful indirect marker of reservoir size/activity for monitoring viral persistence in eradication and treatment intensification studies.

**PP 3.15**

Ultradeep sequencing characterization of HIV-1 diversity in primary infection

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**Background:** HIV-1 diversity during primary infection is associated with viral immune control. We assay the genetic diversity of the envelope V3 region using next-generation sequencing (NGS) in different compartments in patients at the time of primary infection.

**Methods:** Quantification of HIV-1 DNA in peripheral blood mononuclear cells (PBMC, n=7), non-spermatozoid cells (NSC, n=3), and lymphoid cells of the rectal tissue (n=3) was performed in eight patients at the time of primary infection (T/8 were MSM, 1 Fiebig II, 2 Fiebig IV and 5 Fiebig V). HIV-1 RNA in plasma (PL, n=8), seminal fluid (SF, n=8) and saliva (SAL, n=3) was also quantified. NGS of viral DNA and RNA was performed on amplicons (408pb) using Roche/454 GS-Junior. Generated sequences were analyzed by: (a) calculating the maximal diversity index (MDI); (b) reconstructing in silico variants (haplotypes); (c) building Neighbor Joining trees; (d) and searching for compartmentalization using tree and pairwise distance based tests. A threshold of 1% was chosen for considering a haplotype.

**Results:** Median (IQR) HIV-1 DNA was 4.1 [3.1–4.1] log/10^6 PBMC and 3.7 [3.5–3.8] log/10^6 rectal cells. Median HIV-1 RNA was 6.2 [5.5–6.95] log/mL in PL, 4.9 [4.2–5.3] log/mL in SF, and 4.88 [4.4–5.3] log/mL in SAL. For each patient, a median of 4550 sequences per compartments were analyzed. The median MDI per patient varied from 0.7% to 6.6% suggesting a very low diversity, confirmed by the clonal aspect of the phylogenetic trees. A median of five haplotypes (range: 3–19) per compartments was created. In the earliest stages of infection, a major haplotype was present in all compartments, representing more than 70% of the sequences. Four significant viral compartmentalization events were found.

**Conclusions:** Viral dissemination and massive replication occur within the first month after HIV infection. A homogenous clonal viral population in each compartment and between compartments was demonstrated using deep sequencing.

**PP 3.16**

Correlation between HIV-2 RNA and HIV-2 total DNA levels

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**Background:** Few data are available regarding HIV-2 reservoir. The aim of the study was to assess the size of reservoir in different populations of HIV-2-infected patients.

**Methods:** HIV-2 total DNA was assessed in ARV-naive and ARV-treated patients in virological failure (VF) included in the HIV-2 ANRS CO5 cohort. HIV-2 total DNA and RNA quantifications were performed using "in-house" real-time PCR assays: adapted from the HIV-2 RNA Biocentric kit with a LOQ=7.5 copies/PCR for DNA and with a LOQ=100 copies/mL for RNA.

**Results:** Among the 57 ARV-naive patients, median CD4-cell-count was 475/mm^3 (IQR=381–659), plasma viral load (pVL) and HIV-2 total DNA were below the LOQ in 74% and in 12% of the patients, respectively. Median pVL was 1,458 copies/mL (IQR=313–3,574); median HIV-2 total DNA was 2.7 log_{10} copies/10^6 PBMC (IQR=2.5–3.2). No difference was observed in HIV-2 total DNA or RNA values between patients infected with group A and with group B (P=0.22; P=0.06).

Among the 50 patients with VF, treated since a median of 8 years (IQR=4–13), median CD4-cell-count was 232/mm^3 (IQR=137–361), 14% and 2% had pVL and HIV-2 total DNA below the LOQ, respectively. Median pVL was 832 copies/mL (IQR=192–4,011) and median HIV-2 total DNA was 3.2 log_{10} copies/10^6 PBMC (IQR=2.6–3.5). HIV-2 total DNA and RNA values were significantly higher in patients infected with group A than with group B (3.3 vs. 2.7 log_{10}
levels: r=0.74, CI95%=0.57–0.85. In ARV-treated patients, a trend to a lower proportion of patients with HIV-2 total DNA below the LOQ and a significant higher median
In ARV-treated patients, a trend to a lower proportion of patients with HIV-2 RNA and DNA levels. The size of reservoir was significantly higher in ARV-treated patient in VF than in ARV-naïve patients, as described in HIV-1 infection.

PP 3.17
Integrated and total HIV-1 DNA can predict quantitative viral outgrowth in patients on long-term ART
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Background: Accurate markers to assess the replication competent HIV reservoir are urgently needed to support HIV-1 cure efforts. Despite the development of diverse PCR and cell culture-based assays, few comparative studies have been performed on patient samples.

Methods: We designed a study to evaluate and compare HIV-1 reservoir markers in 25 patients. These were selected from a previous study (n=164) in order to select patients with a high, intermediate or low HIV-1 burden, based on total HIV-1 DNA and cell associated HIV-1 RNA. Patients were on ART for a median of 9 years (IQR 6–12). Total and integrated HIV-1 DNA, unspliced (us) HIV-1 RNA, and 2LTR circles were quantified by digital PCR in peripheral blood, and a viral outgrowth assay (VOA) was performed.

Results: The VOA correlated with integrated HIV-1 DNA (P=0.05, R²=0.44) and total HIV-1 DNA (P=0.019, R²=0.54), but not with usHIV-1 RNA, nor with 2LTR circles. Integrated HIV-1 DNA correlated with usHIV-1 RNA (P=0.001, R²=0.28) and total HIV-1 DNA (P=0.002, R²=0.85). Bland Altman analysis to assess the agreement between the assays revealed a quantification bias of the VOA at 2.88 log (95% CI 1.91–3.85) and 2.23 log (95% CI 1.20–3.27) compared to integrated and total HIV-1 DNA, respectively. However, this bias was not constant, as the difference between both methods changes with the reservoir size. Using the Bland Altman model, we propose a prediction model to predict viral outgrowth output from HIV DNA measures in patients on ART.

Conclusion: Our study reveals important correlations between the VOA and HIV-1 DNA measures, suggesting that the total pool of HIV-1 DNA may predict the size of the replication competent virus in ART suppressed patients. However, this study was restricted to patients on long term ART who started treatment during chronic HIV infection. Future studies should investigate whether HIV DNA and the VOA also correlate in early treated patients or after reservoir purging interventions.

PP 3.18
A theoretical framework to guide clinical trial design to estimate efficacies of latency-reversing agents
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Background: Recently, HIV eradication studies have focused on developing latency-reversing agents (LRAs) to activate HIV expression in latently infected cells and ultimately purge the HIV latent reservoir. However, measuring the size of the latent reservoir reduction experimentally has been challenging, and it is not clear which steps in the latency-reversing processes determine the rate of latent reservoir reduction. This makes evaluating the efficacy of candidate latent reversal agents and predicting long-term treatment outcomes difficult.

Methods and Results: We constructed a mathematical model to describe the dynamics in latently infected cells under LRA treatment. Using the model, we show that the rate of latent reservoir reduction is strongly dependent on three key parameters: the rate at which latently infected cells become activated, the rate at which LRA activated cells return to latency and the death rate of LRA activated cells. We further extended the model to describe clinical sampling procedure and examined the accuracy of estimating the three key parameters using data collected from three commonly used assays: the viral outgrowth assay, q-PCR to measure cell-associated unspliced HIV RNA and q-PCR to measure plasma virus. We show that parameter estimation based on the viral outgrowth assay is unreliable due to stochastic variations in the low number of latently infected cells in a clinical sample. The q-PCR measurement of plasma viremia provides the best estimates, if the rate of viral production in LRA activated cells is at least 5% of the rate in productively infected cells. We further explore how combining data from multiple assays and increasing assay accuracy would improve estimation of the key parameters.

Conclusions: The theoretical framework we have developed could be used in designing future clinical trials and experiments to evaluate the efficacy of candidate latency-reversing agents and predict long-term treatment outcomes.

PP 3.19
Evidence of ongoing HIV replication during suppressive antiretroviral therapy
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Background: HIV-1 genetic diversity and divergence result from replication in the setting of immune pressure. Antiretroviral therapy (ART) is proposed to abrogate viral genetic evolution when it achieves undetectable viral load. We hypothesized that current ART regimens are not fully suppressive and allow HIV genetic evolution to proceed.

Methods: Cell-associated HIV gp41 DNA was ultra-deep sequenced from 12 HIV-infected individuals pre-ART, and 4 years post continuous suppressive-ART. Trimmed filtered reads were analyzed using QuRe software to reconstruct viral variants, which were subject to phylogenetic analysis. HIV population genetics at each time point was analyzed using measures of divergence and diversity based on pairwise distance, and selection pressure was inferred using dN/dS tests.

Results: There was no correlation between pre-ART viral load and number of sequence reads (P=0.54). There was a positive correlation between number of reconstructed viral lineages and pre-ART viral load (P=0.01) but no correlation with the number of reads (P=0.44). Phylogenetic analysis revealed that HIV diversity was similar between time points despite several years of ART (pre-ART range 0.006–0.04, post-ART range 0.003–0.03; mean pre-ART vs. post-ART: 0.014 on 0.015 repeated measures t-test P=0.2). Viral divergence during ART with respect to baseline approached statistical significance (pre-ART range 0.007–0.02, post-ART range 0.007–0.16; mean pre-ART vs. post-ART: 0.014 vs. 0.038 repeated measures t-test P=0.07). dN/dS analysis showed lack of selection pressure (mean ratio pre-ART 1.17, post-ART 1.1 repeated measures t-test, P=0.7) despite high range observed (pre-ART 0.27–2.37; post-ART 0.14–3.61).

Conclusions: High HIV replicative rates as inferred by pre-ART viral loads relates to a higher number of reconstructed viral lineages
and increased viral divergence after ART suppression, suggesting that at least in some cases, ART treatment is not completely suppressive.

**PP 3.20**

Peripheral blood CD4+ T cells and intestinal lamina propria mononuclear cells contribute to viremia following an analytical treatment interruption: a follow-up analysis of the panobinostat trial

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**Methods:** CD4+ T cells were obtained before, during, and after panobinostat administration (n=15). Five participants participated in an ATI and donated LPMCs. We used single-proviral/gene dispensing to determine the genetic composition HIV-1 DNA and RNA. Phylogenetic analyses were conducted using MEGA 6.0.

**Results:** We identified an expansion of clonal cell-associated HIV-1 DNA in the peripheral blood, which is indicative of previous proliferative fractions that matched viral RNA sequences from the ATI. Cell-associated HIV-1 DNA sequences from CD4+ T cells from both blood and intestinal lamina propria mononuclear cells (LPMCs) during panobinostat therapy to sequences collected from the plasma during a post-panobinostat analytical treatment interruption (ATI).

**Conclusions:** Clonally expanded HIV-1 is capable of contributing to viremia. Importantly, we found that cell-associated HIV-1 DNA in the blood and LPMCs contribute to plasma viremia following discontinuation of ART. Furthermore, HIV-1 RNA from virus that later emerged during ATI was expressed in both tissues during panobinostat treatment. Thus, both of these important reservoirs of latent virus should be prioritised in remission and curative strategies.

**PP 3.22**

Diversity changes in blood HIV-1 DNA reservoir after combination of chemotherapy and autologous hematopoietic stem cell transplantation for lymphoma

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**Background:** After high-dose chemotherapy and autologous stem cell transplantation (HDC/ASCT) for lymphoma in HIV-1-infected patients, viral DNA persists in PBMCs, but reservoir diversity has not been assayed so far.

**Methods:** Longitudinal blood samples from 13 retrospective HIV-1-infected lymphoma patients treated with HDC/ASCT in Hôpital Saint Louis, Paris were assayed for HIV-1 DNA (HIV DNA Cell, Biocentric). Samples from two patients suppressed on 3TC-ABC-DRV/r with relapsed DLBCL were selected for ultra-deep sequencing (UDS) of 373bp region in gp120 (454GS Junior, Roche) at two time points before and after HDC/ASCT. Viremia was investigated by neighbour-joining (NJ) analysis (MEGA, 1000 bootstraps), time-scaled maximum clade credibility (MCC, Beast) and compartmentalization tests.

**Results:** 85 samples (median 7/patient) were assayed for HIV-1 DNA and did not display overall nor patient-specific quantitative changes in blood reservoir levels before and after HDC/ASCT (median 2.88 [2.44–3.20] log10 copies vs. 2.56 [2.09–2.73] log10 copies/106 PBMC, respectively). UDS of gp120 in two patients yielded ≈10,000 sequences/sample, clustered into haplotypes for further analysis. In one patient infected with CRF02_AG who started cART 7 months before HDC/ASCT, overall diversity decreased but there was no evidence of a shift in reservoir HIV-1 populations in PBMCs over time. Alternatively, in a patient infected with subtype B and on cART for 7 years before ASCT, haplotypes from each time point were distinct in NJ and MCC, and post-ASCT haplotypes did not descend from pre-ASCT time point 1.

**Conclusion:** Although blood HIV-1 DNA quantification rises quickly back to its former level after HDC/ASCT while on cART, pointing at homeostatic proliferation from autologous infected grafts and tissue reservoirs, UDS of proviral populations in blood unveiled qualitative changes. This shift in diversity supports the ability of high-dose chemotherapy to notably alter HIV-1 DNA reservoir.
PP 3.23

Abstract withdrawn

PP 3.24

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

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It has been suggested that HIV-1 by integrating near cancer-associated genes could promote the expansion and persistence of infected cells in patients under antiretroviral therapy (ART). However, the molecular mechanism/s of insertional mutagenesis used and the physiological impact on the cells harboring these integrations are completely unknown.

Here, we found that in peripheral blood mononuclear cells (PBMC) from 54 HIV-1 infected patients under ART, BACH2 and STAT5B were targeted by a significantly higher number of integrations (P<0.0001) and with the same orientation of gene transcription compared to other lentiviral integration datasets. Furthermore, aberrant chimeric transcripts containing viral sequences fused to the first protein coding exon of BACH2 or STAT5B and predicted to encode for unaltered full-length proteins were found in PBMC of 34% of HIV-1 patients under ART (30/87). Tracking the expression HIV-1/STAT5B transcripts in purified T cell subpopulations and monocytes (n=6) we found a specific enrichment of chimeric HIV/STAT5B mRNAs in T-regulatory (reg) and T-central memory (cm) cells in all patients tested (n=6).

In vitro experiment on CD4+ T-cells isolated from healthy donor showed that forced expression of these transcription factors significantly increased their proliferation rate and do not alter the immune-suppressive function of T-reg cells.

Hence, our findings provide novel evidence that HIV-1 takes advantage of insertional mutagenesis to favor its persistence in the host by activating STAT5B and BACH2. Indeed, the selective advantage conferred by these integrations should favor the survival and proliferation of T-reg and T-cm cells which are long lived, potentially able to diminish the immune surveillance against infected cells thus favoring long-term viral persistence.

PP 3.25

Diverse proviral structure of HIV integrants in clonally expanded cells

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Background: Clonally expanded populations of HIV infected cells persist during prolonged ART. Recently, we identified several highly expanded lineages, including a clone with an integrant in the HORMAD2 gene that accounted for c. 50% of all of the infected cells.

Integration into specific introns of BACH2 or MKL2 increased the expansion and/or survival of infected cells. In one instance a clonally expanded provirus was infectious. The proviruses in these clonally expanded cells have not been adequately characterized. We describe characterization of other proviruses in highly expanded clones.

Methods: Longitudinal PBMC samples were obtained from volunteers with chronic HIV infection. Integration sites were determined as previously described. Proviruses integrated in HORMAD2, MKL2, and an intergenic region of the X chromosome were selectively amplified using specific primers for HIV and the flanking host DNA.

Results: The HORMAD2 integrant was present both pre- and on-cART and accounted for 50–80% of all of the infected cells after 7–8 years on cART, implying that expansion of the clone started early in infection. Proviral sequence analysis revealed a 675 nt single LTR with intact promoter elements. Analysis of a highly expanded provirus in an intergenic region of the X chromosome also revealed evidence for a solo LTR. Analysis of seven integrants in MKL2 were also characterized. One of the proviruses had a large pol–U3 internal deletion; of the remaining, four proviruses showed evidence CtoA mutation leading to multiple stop codons and three were found to lack intact tat or rev. All seven, however, had intact LTR promoter elements and retained the major splice donor sequence.

Conclusions: We found proviruses in expanded clones that were intact, hypermutated, partially deleted or consisting of a solo LTR. MKL2 proviruses, have intact LTR regulatory elements but some lack tat, which is normally required for HIV transcription.

PP 3.26

No selection of CXCR4-using variants in cell reservoirs of dual-mixed HIV infected patients receiving suppressive maravirocy therapy

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Background: The CCR5 antagonist maraviroc is only active against CCR5-using viruses. However, some patients were given maraviroc although they were infected by R5X4 dual-mixed viruses. In the MARIMUNO study, the patients received a 24-week maraviroc supplement to an efficient antiretroviral therapy. Thus, we investigated how the frequency of CXCR4-using variants in R5X4 dual-mixed virus populations responded to maraviroc selection pressure using ultra-deep sequencing (UDS).

Methods: We explored 22 patients from the MARIMUNO study infected with R5X4 dual-mixed viruses according to the recombinant virus assay Toulouse Tropism Test. The frequency of CXCR4-using variants was determined in peripheral blood mononuclear cells (PBMCs) before maraviroc intensification (week 0) and after 24 weeks of maraviroc (week 24). UDS was performed on a 454 GS Junior system. The sequence reads of the V3 env regions were analysed with the PyroVir software developed to provide an automated position-specific process for inferring HIV-1 tropism from V3 env UDS data.

Results: The mean total HIV-1 DNA was stable between week 0 and 24 (2.4 log copies/10^6 cells and 2.5 log copies/10^6 cells, respectively). UDS with the PyroVir genotypic algorithm detected CXCR4-using viruses in the 22 R5X4 infected patients at week 0 with a mean frequency of 59% (range: 3–100%). CXCR4-using viruses were detected in 21/22 patients at week 24 with a mean frequency of 52% (range: 10–92%). We found no correlation between the HIV DNA concentration in PBMCs and the number of CXCR4-using variants or their frequency. The frequency of CXCR4-using variants did not increase between week 0 and 24 except in one patient.

Conclusions: A 24-week course of a CCR5 antagonist does not select CXCR4-using viruses in the PBMCs of patients on suppressive therapy infected with R5X4 dual-mixed viruses. These results indicate little or no residual HIV replication that could be subjected to selection pressure.

Session 4: Anatomic and non-CD4 cell reservoirs

PP 4.0

Kinetics of HIV production by monocyte derived macrophages (MDM) in the presence of erythrocytes

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Background: Erythrocytes (E) of HIV+ individuals carry HIV RNA and p24Ag even if the viral load is undetectable. Macrophages are in close contact with E and therefore studying the interaction between these cell types is highly relevant. Our previous studies demonstrated that the presence of E enhances viral production in HIV infected MDM cultures. This work focuses on the kinetics of MDM HIV production in presence of E obtained from HIV+ and HIV+ individuals.

Methods: MDM were obtained from healthy donors and were infected with the HIV-1 Bal strain. E from HIV+ and HIV+ individuals were purified with Dextran. Fourteen days p.i. MDM were incubated with E or RPMI (as control) for 5, 15, 30 and 60 minutes. Viral production in MDM cultured with E was evaluated by measuring p24Ag in the culture supernatant plus p24Ag associated to E.

Results: Highest viral production in control culture was observed at the 30’ timepoint, while a significant decay was detected at the 60’ timepoint. Viral production in the presence of HIV-E reached its maximum increase at the 15’ timepoint with no later decay. When viral production of MDM in the presence of E vs. control were compared, a maximum difference (6-fold increase) was observed at the 15’ timepoint (P<0.035). The amount of virus produced when MDM were incubated with HIV+E was significantly higher than that produced in the control culture (P<0.005).

Conclusions: Our results highlight the impact of erythrocytes in viral replication kinetics of HIV-1 infected MDM. As shown, viral load was greater at early timepoints when E were present in the MDM culture, suggesting that this cell type may facilitate viral budding/release. This effect was evident regardless the erythrocyte origin (HIV+E or HIV-E), indicating that erythrocytes have an important role in the pathogenesis of HIV infection in physiologic conditions.

PP 4.1

Levels of SAMHD1 and natural ribonucleotides may alter anti-HIV potency of antiretroviral agents in primary CD4+ CCR5+ placental macrophages

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Background: Perinatal transmission of HIV remains a significant global health concern with ~200,000 new infections worldwide in exposed infants. Cellular protective mechanisms of antiretroviral agents (ARV) administered during pregnancy remain unclear. Understanding the pharmacology and potency of ARV in placental macrophages or Hofbauer cells (HC), a potential HIV reservoir, provides an opportunity to elucidate the dynamics between ARV and reservoir sites. Herein, antiviral potency of clinically relevant ARV and cellular factors impacting viral replication/potency, including dNTP/rNTP and SAMHD1 levels, were assessed in resting and activated HC and compared to adult macrophages.

Methods: HC or monocytes were isolated from healthy donors (placenta or buffy coat). Macrophages were differentiated with GM-CSF for 7 days. Activated or resting cells were maintained with or without GM-CSF, respectively. Cells were treated with various concentrations of ARV for 2 hours prior to infection with HIV-1a, and maintained for 6 days before viral quantification (p24-ELISA). LC-MS/MS was used to quantify dNTP/rNTP levels. SAMHD1 levels were quantified by FACS.

Results: Antiviral potency of NRTI significantly (P<0.01) diminished in activated versus resting HC (EC50 0.01–0.9 μM versus 0.001–0.01 μM), while antiviral potency of non-nucleoside reverse transcriptase, integrase, or protease inhibitors, remained constant. Intracellular levels of dNTP were similar in HC and macrophages, however rNTPs ATP, CTP, and TTP levels were significantly higher in resting HC versus resting macrophages (7–10 fold, P<0.01). CTP and UTP were significantly higher in activated HC versus activated macrophages (3–7 fold, P<0.05). HC expressed high levels of SAMHD1 independent of activation state, whereas activation increased SAMHD1 levels in macrophages.

Conclusions: rNTPs were significantly higher in HC versus macrophages potentially reflecting increased incorporation in these cells. SAMHD1, which degrades dNTPs, is high in HC independent of activation state, which may account for increased ratios of rNTP:dNTP in HC. Cellular activation significantly decreased potency of ARV in HC, underscoring necessity for antiviral agents targeting virus intracellularly. High rNTP in HC coupled with high SAMHD1 levels (which confer dNTP degradation), suggest that ribonucleoside inhibitors should be explored as novel antiviral agents to exploit this unique cell type.
Inclusion criteria were undetectable viral load and stable treatment for at least 6 months. Patients were divided into: unimpaired, asymptomatic neurocognitive disorders (ANI) and symptomatic HAND (sHAND), represented by mild neurocognitive disorders (MND) and HIV-associated dementia (HAD). Demographic and background parameters, immune activation markers and CD4/CD8 ratio values were recorded. A cross-sectional analysis of parameters associated with NP test results was performed.

Results: 204 patients were included (mean age 52 years, 78% male, mean CD4 620, nadir CD4 240, 28% HCV-co-infected, 16 years of infection, 2.9 years on current treatment). HAND counted for 30% of subjects, including 20% ANI and 10% sHAND. Among patients with sHAND, 80% had MND and 20% HAD. In multivariate analysis the CD4/CD8 ratio <1 was associated with CD4 nadir <200 (OR 3.14) and higher CD4+CD38+HLA-DR+ cells (OR 1.24). Logistic regression showed that patients with ANI or sHAND were older (OR 1.05 and 1.08, respectively) than unimpaired subjects, while those with sHAND had higher risks to be HCV co-infected (OR 3.97) and have a CD4/CD8 ratio below 1 (OR 11.2). Patients with ANI diverged from unimpaired subjects only for a lower education level (OR 2.71).

Conclusions: Aviremic patients with sHAND, but not those with ANI, have higher immune activation than unimpaired subjects, suggesting different pathophysiological mechanisms.

PP 4.3
Asymptomatic cerebrospinal fluid viral escape during ART is associated with increased intrathecal immune activation
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Background: Asymptomatic cerebrospinal fluid (CSF) viral escape, where HIV-1 RNA is increased in CSF while suppressed in plasma, occurs infrequently in patients on effective antiretroviral therapy (ART). It is unclear if CSF escape is benign or represents an active CNS infection. We examined the relationship between asymptomatic CSF viral escape and CSF biomarkers of axonal injury (the light subunit of sHAND, 80% had MND and 20% HAD. In multivariate analysis the CD4/CD8 ratio <1 was associated with CD4 nadir <200 (OR 3.14) and higher CD4+CD38+HLA-DR+ cells (OR 1.24). Logistic regression showed that patients with ANI or sHAND were older (OR 1.05 and 1.08, respectively) than unimpaired subjects, while those with sHAND had higher risks to be HCV co-infected (OR 3.97) and have a CD4/CD8 ratio below 1 (OR 11.2). Patients with ANI diverged from unimpaired subjects only for a lower education level (OR 2.71).

Conclusions: Aviremic patients with sHAND, but not those with ANI, have higher immune activation than unimpaired subjects, suggesting different pathophysiological mechanisms.

PP 4.4
HIV isolated from CSF cells of a virologically controlled patient infects astrocytes
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Background: In vivo studies show that astrocytes may be a critical reservoir for HIV in the brain. However, in vivo infection of astrocytes in vitro is inefficient. We investigated if HIV strains from the central nervous system were able to infect astrocytes.

Methods: We collected CSF cells via lumbar drain from six patients with plasma viral suppression; five had normal cognitive performance and one had HIV-associated neurocognitive disorder (HAND). HIV was not detected in CSF of all patients by clinical lab. We isolated an HIV virus from the HAND patient after 10 days coculture with PBMCs from normal donors. This virus was able to infect MT2 cells and Jurkat-Tat (JKT) cells and significantly induced syncytia, but could not infect THP-1-derived macrophages. These observations indicate that the isolate was an X4-tropic virus. When the virus stock was initially incubated with astrocytes, no infection was detected. However, the virus could infect astrocytes via the transwell culture system where HIV-infected JKT cells were loaded on the top chamber and only HIV particles could reach the astrocytes. This is consistent with previous observations that immature HIV particles released from the infected lymphocytes were able to directly bind to CXCR4 on astrocytes in the absence of CD4 triggering virus-cell fusion and leading to infection. We have confirmed this mechanism in the cell-to-cell infection of HIV in astrocytes by cocultivating with the infected lymphocytes.

Conclusions: Despite adequate antiretroviral therapy low level HIV may be present in the CSF that can infect lymphocytes and astrocytes.

PP 4.5
CCR5- and CXCR4-tropic HIVs infect CD4+ hematopoietic stem and progenitor cells in vitro and in optimally treated people
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Hematopoietic stem and progenitor cells (HSPC) express HIV receptors and support both active and latent infection. To determine the co-receptors used by HIV to infect HSPCs in vivo, we sampled bone marrow from HIV+ donors with undetectable viral loads for at least 6 months. We purified two populations of HSPCs based on CD133 expression (CD133high and CD133low) as CD133 marks a subset of CD34 HSPCs; however, we isolated CXCR4-tropic subtype B env amplicons from both CD133high and CD133low HSPCs. However, we isolated CXCR4-tropic subtype B env amplicons solely from the CD133low subpopulation. In vitro, CXCR4- and CCR5-tropic Env-pseudotyped viruses preferentially transduced CD4low over CD4high HSPCs. In addition, while viruses of both tropisms infected CD133high cells, we confirmed that CXCR4-tropic viruses infected cells with the highest amount of CD133 more efficiently than CCR5-tropic viruses. To assess the significance of differential CD4 and CD133 expression, we used cell surface markers that distinguish subsets with different developmental capacity (CD38, CD45RA and CD10). CD133+/CD45RA-
cells contained the highest fraction of stem cells and multipotent progenitors, CD133<sup>SP</sup>CD4<sup>int</sup> and CD133<sup>low</sup>CD4<sup>high</sup> cells contained intermediate fractions, and CD133<sup>neg</sup>CD4<sup>neg</sup> HSPCs contained the smallest fraction. We also found that CD133<sup>SP</sup>CD4<sup>int</sup> cells were enriched in differentiated lymphoid and myeloid progenitors. In sum, our data indicate that CD4 marks an HSPC subset that is differentiated lymphoid and myeloid progenitors. We also found that CD133<sup>high</sup>CD4<sup>low</sup> cells were enriched in intermediate fractions, and CD133<sup>low</sup>CD4<sup>low</sup> HSPCs contained the smallest fraction of stem cells and multipotent progenitors. Most tissues of a plasma-negative HIV autopsy cohort contain HIV DNA and many exhibit tissue pathology. The high rate of comorbidities in ART+HIV+ patients suggests that viral persistence in anatomical sites may contribute to tissue pathology. We identified subjects with undetectable viral load at death, assayed a diverse panel of their autopsy tissues for HIV, and assessed tissue histopathology to discover if residual anatomical HIV levels during cART were potentially related to tissue injury. A subset of tissues was assessed for HIV RNA using an in situ hybridization assay.

**Results:** The mean patient age and span of HIV infection was 46.5 years and 12 years, respectively. Fifteen of the 20 patients developed cancer. Abnormal pathology was identified in the spleen, lung, lymph node and liver in 90% of the patients. Aorta and kidney were abnormal in 50 and 60% of the participants, respectively. 75% of participants exhibited atherosclerosis and all brain tissues exhibited slight to severe pathology. 66% of the tissues studied contained HIV DNA copies >200 million cell equivalents. Hybridization studies localized HIV RNA to macrophages within cancer tissues. CD68/CD163+ macrophages surrounded HIV+ cells in the brain. Residual HIV within diverse anatomical tissues could promote inflammatory diseases, including cancer, atherosclerosis and other organ-associated diseases. These ACSR/NNAH cohorts, along with others of their kind, are highly valuable resources for future studies of HIV reservoirs and persistence.

Curing HIV infection requires the eradication of all infected cells; recent studies suggest that hematopoietic stem and progenitor cells (HSPCs) may serve as a reservoir in vivo. We collected bone marrow and peripheral blood from 46 cART treated HIV-1 infected people with undetectable viremia for at least 6 months, including two donors treated in acute phase. We PCR amplified gag and env from highly purified HSPC, PBMC and plasma virus and recovered HIV-1 amplicons from 31 donors. For 23 of 27 donors tested, including a donor treated since acute infection, the frequency of provirus detected in HSPCs was higher than would be expected from T cell contamination (P<0.01). We examined the reproducibility of provirus detection with four participants who donated multiple times at 3–6 month intervals. We found provirus in HSPC from all three donations from one donor and from both donations from two donors. We detected provirus in HSPC from one of two donations from the fourth donor, but could not rule out T cells as the source of HIV in one donation. Thus far, we have generated cDNA from the plasma of 23 donors and recovered HIV sequences from 16. Twelve donors yielded amplifiable HIV sequences from both HSPC and plasma virus. Three of these donors had plasma viral sequences that were identical to provirus from highly purified HSPCs. One donor had a cluster of clonal plasma viral sequences that matched an HSPC-associated provirus. In ongoing studies, we have infected T cell lines with reporter viruses pseudotyped with full-length Env amplicons isolated from HSPC. In sum, provirus can be detected in HSPC isolated from a large subset of optimally treated, HIV-infected people, including one person treated since acute infection. Moreover, some HSPC proviruses show sequence identity to circulating plasma virus and have functional Env. These data support HSPCs as a reservoir of HIV-1 in optimally treated HIV-infected people.

**Session 5: Immunology of HIV persistence**

**PP 5.0**

*Modulations in key defense responses of epithelial cells during co-stimulation with BCG and HIV-Nef*

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**Background:** Pulmonary tuberculosis (being the most common form) is an infectious disease that carves its way to the lungs through the respiratory route but it can very well spread to other organs. Considering the residence of M. tb as lungs, epithelial cells are one of the foremost host cells interacting with the invading pathogen. In this study we used epithelial cells to understand the mechanism that modulates innate immunity during co-infection of HIV with BCG.

**Method:** A549 epithelial cells were used in this study. Cells were stimulated with HIV-1 Nef or infected with (2 MOI) BCG or both for 24 h. ROS analysis was performed using DCDHA for 30 min, 60 min and 120 min time points through Flow cytometry. Annexin V assay was performed to monitor the apoptosis. Expression of NF-kB and autophagy molecules such as BECN 1and LC-3 was monitored using western blotting. Expression level of various co-stimulatory molecules was monitored using flow cytometry.

**Results:** Our results show that HIV-1 early protein Nef downregulates BCG mediated ROS and co-stimulatory molecule CD80. Further decrease in Annexin V staining was observed upon stimulation with BCG, Nef or BCG and together. Interestingly, we found that the expression of key mediators of autophagy, Beclin1 and LC3, significantly decrease when epithelial cells are challenged with BCG and Nef together.

**Conclusion:** Collectively, our results indicate that HIV-1 Nef and BCG synergistically inhibit epithelial cell apoptosis and autophagy. Our study points out the mechanisms employed by these pathogens to evade epithelial cell defense mechanisms during co-infection.
PP 5.1

HIV alters the profile of cytokines responding to seasonal influenza vaccination
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Background: The aim of this study was to assess how influenza vaccine responses in HIV+ and age matched HIV- individuals correlate with age and a curated panel of inflammatory biomarkers.

Methods: 281 participants [HIV+: n=131; HIV-: n=150] aged 18–83 years were given TIV during the 2013–14 or 2014–15 flu season. Blood was collected pre-vaccination (T0) and at day 7 (T1), 21 (T2) and 180 (T3) post-vaccination. Antibody responses to flu vaccine ags were assessed by hemaglutination inhibition assay (HIA). Vaccine Responders (R) were defined as those achieving a 4-fold increase in HA Ab titer at T2 relative to T0; those who failed were Non-Responders (NR). Plasma was assessed for 15 cytokines, three markers of microbial translocation, and three markers of cardiovascular inflammation by Luminex Multiplex bead assay or ELISA.

Results: HIA titer to H1N1 Ag at T0 showed seroprotection (>1:40) in 69% HIV- and 74% HIV+ (range 80–2560). Because of low frequency of R when T0 titer was high (>1:360) we selected participants with T0 titer of ≤360 (HIV-: n=126, R 53%; HIV+: n=100, R 38%). In HIV-, age was inversely correlated with Ab titer at T0 (R=0.004) and T2 (R=0.001) whereas in HIV+, there was no correlation of age with T0 Ab titer but an inverse relationship was evident for T2 titer (R=0.043). Among HIV-, age was positively correlated with T0 IL-17A, MCP-1, TNF-α, CRP, Neopterin, and sCD163 (P=0.05 for all). However, the age-related inflammatory profile in HIV+ showed T0 IL-8, sVCAM, CRP, Neopterin, sCD163, and sTNFRII (P=0.05 for all) were significant. In HIV+ NR, the cytokines IFNα2 and IL-8 were negatively associated with Ab titer at T0 (R=0.010 and P=0.050, respectively) and T2 (R=0.004 and P=0.022, respectively) whereas in HIV+ or HIV- this association was not observed.

Conclusions: HIV+ and HIV- show different age-related baseline cytokine profiles and a cytokine signature of responsiveness or unresponsiveness was evident suggesting viral persistence plays a role in flu vaccine responses.

PP 5.2

Virologic and immunologic correlates of viral control after ART-interruption in SIV-infected rhesus macaques
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Background: ART does not eradicate HIV and the virus rebounds upon ART interruption. A sustained control of HIV replication off-ART has been described in a subset of subjects starting ART early after infection (post-treatment controllers; PTC). The determinants of post-ART HIV control are still unclear, particularly in tissues. Here, we used SIV-infected rhesus macaques (RM) to investigate the features associated with post-ART SIV control.

Methods: We identified five SIV-infected RM that, after 7 months of ART started at 2-months p.i., controlled viral rebound (<200 copies/mL) after structured treatment interruption (STI). Blood (PB), rectum (RB), and lymph node (LN) samples were collected from these animals and from RMs that, under the same conditions, experienced a robust SIV rebound after STI (non controllers; NC). Total SIV-DNA and RNA was measured on PB CD4 T cells and gut tissues by qPCR; immunological parameters were determined by flow cytometry. Predictors associated with PTC status were evaluated by odds ratio analyses.

Results: At pre-ART, PTC had reduced SIV-RNA levels in plasma and PB, lower SIV-DNA content in PB CD4 T cells and RB, reduced T-cell activation, and higher CD4 counts than NC (P<0.01 for all). Gut CD4 T cells were similar, but PTC had higher frequencies of Th17 cells. On-ART, PTC had significantly lower residual viremia (<3 copies/mL) and SIV-DNA content in PB CD4 T cells. SIV-specific CD8 T cell compartment was comparable between the two groups. Remarkably, PB and RB SIV-DNA contents rapidly increased in NC after ART interruption, while remain stable or progressively decreased in PTC. Finally, partial control of SIV rebound in PTC resulted in higher CD4 levels and reduced inflammation during the entire off-ART period.

Conclusions: Lower pre-ART viremia, cell-associated SIV-DNA, and T cell activation with concomitant preservation of Th17 cells are highly associated with prolonged viral control post-ART interruption in SIV-infected RMs.

PP 5.3

Administration of panobinostat is associated with increased IL-17A mRNA in the intestinal epithelium of HIV-1 patients
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Background: Intestinal CD4+ T cell depletion is rapid and profound during early HIV-1 infection. This leads to a compromised mucosal barrier that prompts chronic systemic inflammation. The preferential loss of intestinal Th17 cells in HIV-1 disease is a driver of the damage within the mucosal barrier and disease progression that is not reversed during ART. Given the pathogenesis of HIV-1 in the intestines, understanding the effects of new therapeutic strategies within this organ is a priority.

Methods: We conducted a single-arm, phase I/II clinical trial designed to evaluate the therapeutic effect of the histone deacetylase (HDAC) inhibitor panobinostat on HIV-1 persistence despite successful ART (NCT01680094). We examined colonic biopsies collected before and during oral panobinostat treatment from nine individuals for the effects of panobinostat on viral DNA (ddPCR) and viral RNA (RNAscope ISH). Given that HDAC inhibitors have broad effects beyond virus reactivation (e.g. modulation of immune pathways), we also examined intestinal T cell activation (flow cytometry) and inflammatory cytokine mRNA production (RNAscope ISH).

Results: As reported for peripheral blood cells from these patients, there were no panobinostat-associated cohort-wide changes in viral DNA in the intestinal biopsies. Productively infected cells were detected in biopsy sections but were too rare for quantification. We observed a decrease in the frequency of intestinal lamina propria T cells expressing the activation marker CD69. Notably, we observed an increase in IL-17A mRNA expression specifically within the intestinal epithelium that was associated with panobinostat treatment (P<0.04).

Conclusions: Panobinostat had a clear biological impact in the intestines of HIV-1 patients as shown by the decreased activation and increased IL-17A expression in the intestinal epithelium. These results suggest that panobinostat therapy may influence the restoration of mucosal barrier function in these patients.

PP 5.4

Molecular profiling of antigen-specific peripheral T follicular helper cells from HIV-infected donors using influenza vaccination model
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Background: T follicular helper (TFH) cells have been shown to be a preferred cellular subset for HIV reservoirs, however the mechanisms governing HIV persistence in this cell type are unknown. A peripheral
TFH population characterized by expression of the chemokine receptor, CXCR5 has been shown to share some functional properties with GC TFH. The objective of the current study was to evaluate gene expression in H1N1-specific pTFH from HIV-infected and uninfected donors following influenza vaccination.

Methods: Cryopreserved PBMC from HIV-infected (n=6) and uninfected (n=6) individuals 21 days post-influenza vaccination from the 2014–15 flu season were thawed and stimulated with H1N1 antigen (5 ug/mL) overnight (16–18 h) in the presence of fluorescein-conjugated anti-CD40L antibodies. PBMC were stained with antibodies to CD4, CD45RA, CCR7, CXCR5, and a live/dead marker and were FACS-sorted using BD FACs Aria. 50 H1N1-specific pTFH and non-pTFH cells characterized by CD4+CD45RA+CCR7+CD40L+ and CXCR5+ or negative, respectively, were sorted directly into CellsDirect one-step PCR buffer containing primers for specific target amplification. The resulting cDNA was run on Fluidigm BioMark platform to evaluate gene expression of 96 genes using Taqman gene expression assays by RT-PCR. SingularAR, R-based statistical analysis package, was used to compare gene expression from different populations by ANOVA.

Results: H1N1-specific pTFH from HIV-infected donors exhibited significantly (P<0.05) higher expression of genes involved in activation (CD69, IL2RA, STAT1, STAT5A, TRIM5) and immune regulation (FOXP3, TIGIT, ITCH) compared to HIV-uninfected individuals. H1N1-specific (CD40L+) pTFH from HIV-infected individuals also exhibited significantly higher expression of genes, including multiple transcription factors and activation markers compared to CD40L+ non-pTFH. On the other hand, CD40L (neg) pTFH from HIV-infected individuals did not exhibit an activated gene signature and had few differentially expressed genes compared to HIV-uninfected. No differences in the frequency of CD40L+ pTFH were observed between HIV-infected and uninfected vaccines (P=0.59).

Discussion: H1N1-specific pTFH from HIV-infected donors exhibit a gene signature of enhanced activation as well as immune regulation compared to HIV-uninfected. Given the role TFH have been shown to play in maintenance of the HIV reservoir, these data suggest the transcriptional state of activation in antigen-stimulated pTFH may favor HIV replication and persistence.

Co-expression of multiple inhibitory receptors on CD8+ T cells in viremic and ART-suppressed HIV-1(-) individuals

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Background: Inhibitory receptor (IR) expression on CD8+ T cells is associated with regulation of HIV-1-specific function. We tested whether co-expression of multiple IRs on CD8+ T cells in individuals on ART is associated with HIV persistence.

Methods: We evaluated 57 combinations of PD1, 2B4, TIM3, CD160, and CTLA4 in total, naïve (T_N), CD45RA+CCR7+, central (T_C), CD45RA+CCR7+ and effector memory (T_EM; CD45RA+CCR7+) CD8+ T cells in HIV(-) viremic (n=14) and suppressed (n=30) individuals and age-matched HIV(-) controls (n=8) using flow cytometry and Boolean gating. PBMC and plasma were tested for cell-associated HIV RNA (CAR) and DNA (CAD) and residual viremia using qPCR targeting the 3' integrase region of pol.

Results: Suppressed donors had a median CD4+ T count of 642 cells/mm^3 and had been suppressed for a median of 7.4 years. Viremic donors had a median CD4+ T cell count of 240 cells/mm^3 and median HIV-1 RNA of 10500 copies/mL. In HIV(-) and controls, frequencies of total CD8+ T cells co-expressing combinations of 284, 240, 160, and PD1 was higher compared to the %CD8+ T cells that co-expressed combinations including LAG3 and CTLA4 (<0.001; Mann–Whitney). The %CD8+ T cells (total and T_N) expressing 284/CD4160/PD1 was higher in HIV(-) donors (viremics>suppressed) compared to controls (14.25% vs. 6.5 vs. 3.2). %2B4/CD160+ CD8+ T cells (total, T_N, and T_E) was also higher in HIV(-) donors than controls (15% vs. 5.4 vs. 1.4). Despite the higher frequencies in HIV, %2B4/CD160/PD1+ and %2B4/CD160+ CD8+ T cells did not correlate with the levels of residual viremia, CAR, and CAD. %CD8+ T cells expressing other IR combinations were similar in HIV(-) and controls.

Conclusions: 2B4/CD160+ and 2B4/CD160/PD1+ CD8+ T cells are higher in HIV infection despite virologic suppression on ART, but the higher frequencies do not correlate with markers of HIV persistence. Studies blocking multiple IRs are needed to better understand the role of IRs in HIV persistence.
Background: Immune activation in HIV-1-infected patients persists under suppressive antiretroviral treatment and may fuel comorbidities such as atherothrombosis, osteoporosis, metabolic syndrome, neurocognitive disorders and liver steatosis. Our hypothesis was that treated patients present with distinct profiles of immune activation and that each profile is linked to a specific comorbidity. We first explored the profile of immune activation that was associated with the presence of a metabolic syndrome.

Methods: We measured by flow cytometry and ELISA the level of activation of CD4+ and CD8+ T cells, B cells, monocytes, NK cells and endothelial cells as well as of inflammation with a total of 68 soluble and cell surface markers in 120 virologically suppressed individuals and 20 healthy donors (aged ≥45 years). We used a hierarchical clustering analysis to classify the patients according to different markers of immune activation, and logistic regression with odds ratios (OR) and 95% confidence intervals (CI) to measure the association between immune activation profiles and metabolic syndrome.

Results: We observed evidence of inflammation and immune activation in all the cell subpopulations analysed. Patients were clustered in five distinct immune activation profiles. Each one of these five profiles could be characterized by a marker of CD8+ T cell, NK cell, monocyte, endothelial cell activation or of inflammation, respectively, and could be distinguished between the other profiles by a signature of eight biomarkers. Only one of these immune profiles was significantly associated with marks of metabolic syndrome: hypertriglycerideremia (OR 4.18, 95% CI 1.08–16.19, P = 0.038), hyperinsulinemia (OR 12.17, 95% CI 1.79–82.86, P = 0.011) and lipodystrophy (OR 4.87, 95% CI 1.36–17.39, P = 0.015).

Conclusion: We have uncovered an immune signature that might be useful for the prevention and early diagnosis of metabolic syndrome in HIV-infected patients. A better knowledge of the links between immune activation profiles and their consequences might highlight biomarkers predictive of comorbidities, as well as new therapeutic targets in HIV-induced immune activation or other situations of chronic hyperactivity of the immune system including aging.

Session 6: Pharmacology of HIV persistence

PP 6.0

Differential efficacy of antiretroviral drugs in HIV-1 infected human microglia

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Background: HIV-1 infects the human brain within days of primary infection and establishes a viral reservoir. Microglia and perivascular macrophages are productively infected by HIV-1 forming the principal viral reservoir in the brain. The relative susceptibility of this viral reservoir to ART is unknown. We investigated antiretroviral therapy (ART) efficacy in HIV-infected human microglia, bone marrow-derived macrophages (BMDMs) and peripheral blood mononuclear cells (PBMCs). In addition, the concurrent extracellular and intracellular ART concentrations were assessed.

Methods: Human fetal microglia (HFM), and BMDMs were prepared from 16–20 week old fetuses. Peripheral blood mononuclear cells (PBMCs) were also isolated from adult healthy donors. Cultured HFM, BMDMs and PBMCs were infected with HIV-1 at a multiplicity of infection (MOI) ranging from (0.1–1.0). HIV-infected cells were treated with zidovudine (AZT), etravirine (ETR), raltegravir (RAL), darunavir (DRV). The EC50 levels for AZT, RAL, ETR and MVC in PBMCs at day 4 post-infection were: 7.4 nM, 2.7 nM, 2.7 nM and 6.3 nM respectively. Cytotoxicity was not observed for the above ARIs. Exposure of RAL and DRV (100 nM) to differentiated THP1 cells showed that the extracellular concentrations were 63.6 nM and 33.6 nM respectively, while the intracellular concentrations were 4.7 nM and 4.4 nM, respectively.

Conclusions: EC50 values for AZT, ETR and DRV in HIV-infected HFM were exhibited substantially higher than those observed in PBMCs. Intracellular:extracellular concentration ratios were low for RAL and DRV. These results underscore consideration of assessing ART concentrations in different viral reservoirs in efforts to eradicate HIV-1.
Background: Major goals of HIV cure research are to identify latency-reversing agents (LRAs) and to characterize LRAs with unknown mechanism of action (uMOA). The Connectivity Map (CMAP) can address these goals and represents a collection of microarray gene expression profiles generated by treating cell lines with 680 different drug compounds. It is essential to evaluate compatibility between the CMAP (cell lines, microarray and fixed dose) and HIV cure studies (primary CD4 T cells, RNA-Seq, variable doses).

Methods: The following studies were used to assess the utility of the CMAP for HIV cure research: (1) microarray dose response study of primary CD4 T cells from six donors treated with 0, 0.34, 1, 3 and 10 μM of vorinostat; (2) RNA-Seq study of primary CD4+ T cells from four donors treated with 0.34 μM of vorinostat; and (3) differentially expressed genes (DEGs) between latently infected and uninfected cells in the following models: Iglesias-Ussel, Mohammadi, Planelles, Spina, and latently infected cell lines. For all data sets, the top 100 up and down DEGs were queried against the CMAP using gene set enrichment analysis.

Results: The CMAP was able to positively correlate the query profile with vorinostat for studies 1 and 2 when the gene expression data had been generated in primary CD4 T cells (instead of cell lines), by RNA-Seq (instead microarray) and at any dose. The CMAP was then used to identify novel LRAs by searching for negative correlations to the DEGs identified from latency model data. Although the top CMAP hit was different for each model, there were two compounds in common to all five latency models in the top 100 negatively correlated compounds.

Conclusions: CMAP is robust to RNA measuring technique, cell type and dose differences, and therefore represents a useful utility for characterizing LRAs with uMOA. The CMAP may also be used to identify novel LRAs and the compounds identified in this study will be tested for their ability to activate HIV.

PP 7.1

In vitro analysis of different PKC agonists: latency reversal, T-cell activation, cytokine production and isoform selectivity
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Background: In this study, we performed an in vitro analysis of a number of different PKC agonists to determine the PKC isoform selectivity required for HIV latency reversal, T-cell activation potential and cytokine induction. Furthermore, we sought to identify PKC target engagement biomarkers to facilitate in vivo safety studies.

Methods: Nine different PKC agonists were tested in enzymatic assays. EC50 values for latency reversal were calculated using a Jurkat T-cell model of HIV latency. EC50 values were also calculated for prostratin in 2C4 cells lacking specific PKC isoforms. CD69 and CD25 expression in primary T-cells was determined by flow cytometry and a number of inflammatory cytokines measured by multiplex mesoscale analysis.

Results: Nine PKC agonists tested were active in a Jurkat T-cell HIV latency model (EC50 9 nM to 5000 nM). In enzymatic experiments, the majority of PKC agonists tested had activity on PKC isoforms alpha, beta I/II and gamma. However, selective siRNA knockdown of these isoforms had no effect on prostratin-stimulated HIV expression in Jurkat 2C4 cells. A positive correlation was identified between the EC50 required to activate HIV expression in 2C4 cells and the EC50 value for CD69 expression in resting primary T-cells. At these concentrations, we noted significant expression of a number of inflammatory cytokines.

To identify biomarkers of PKC agonism, RNAseq analysis was performed on Jurkat T-cells stimulated with prostratin.

Conclusions: Agonism of multiple PKC isoforms is required for efficient HIV latency reversal in a Jurkat model system. However, among the PKC agonist tested, all elicited CD69 expression and cytokine release at the concentration of latency reversal and cytokine release. In order to facilitate in vivo safety studies, we developed a CD69 mRNA biomarker assay to monitor target engagement and facilitate understanding of desired viral flush versus undesired T-cell activation.

PP 7.2

Suppression of the HIV-1 reservoir with a potent Tat inhibitor
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Despite the success of HIV antiretroviral therapy (ART), HIV persists still in latently and productively infected CD4+ T cells and macrophages in treated individuals. Novel therapeutic agents are needed to limit latent HIV disease.

HIV Tat protein activates transcription and its inhibition blocks viral exponential production. We showed that didehydro-cortistatin A (dCA) binds Tat and potently reduces viral RNA production (EC50 as low as 0.7 pM). dCA was also shown to induce a state of deep latency from which viral reactivation was impaired in cell line models of latency and in latently infected CD4+ T primary cells explanted from ART suppressed individuals, suggesting that the HIV promoter is epigenetically repressed. Using these latter primary cells we developed a latency model and confirmed that viral rebound occurs when antiretrovirals (ARVs) are removed, recapitulating what is observed in patients. In this model prior treatment with ARVs+dCA can almost completely prevent viral rebound up to 21 days when all drugs are removed. Moreover, reactivation with prostratin in the presence of ARVs+dCA was 100% inhibited. Additionally, the number of integrated proviruses in the infected CD4+ T cells decreased in cells treated with ARVs+dCA compared to ARVs alone, suggesting a reduction of cell-to-cell transmission over time.

Macrophages play critical roles in HIV trafficking to the brain contributing to persistence. Access of ARVs to the brain is restricted and HIV immune surveillance inefficient, thus eradication strategies based on viral reactivation might have limitations in this reservoir. We will present results of dCA activity in human primary macrophages infected in vitro.

Our results suggest that dCA combined with ART may abrogate residual HIV production from cellular reservoirs, block viral reactivation, reduce reservoir replenishment, and ultimately decrease the size of the latent reservoir.

Session 9: New therapeutic approaches

PP 9.0

Multi-dose romidepsin in SIV-infected rhesus macaques (RM2s) reactivates latent, replication-competent virus in the absence of antiretroviral therapy (ART)
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Background: Persistent viral reservoirs represent a major obstacle in eliminating HIV-1 from infected individuals. A reservoir reactivation strategy is the “shock and kill” approach, in which latent virus is reactivated with histone deacetylase inhibitors (HDACi) and eliminated through effective CTL responses. Therefore, our goals were to: (1) develop a nonhuman primate model of SIV control with conventional ART; (2) assess the HDACi romidepsin’s (RMD) ability to reactivate SIV in controller RM2s.
Conclusions: Despite activity in Jurkat cells, anti-HIV ADCs failed to demonstrate significant HIV-specific cytotoxicity in primary T-cells. Lack of cytotoxic effect may be related to both the low numbers of gp120 molecules expressed on primary T cells, as well as patient-specific reactivation variations that probably reflect the heterogeneity of the reservoirs and the multiplicity of the mechanisms that underlie HIV latency.
the CRISPR/Cas9 system have been developed to eradicate the HIV-1 genome from infected cells. These results offer a new avenue toward the elimination of HIV-1 to cure HIV/AIDS. CRISPR/Cas9 directed cleavage is mediated through the design of a 20 nucleotide guide RNA (gRNA) which is complementary to the target cleavage site. However, due to the promiscuous nature of the gRNA targeting, it is important to screen for off-target binding sites that may cause unwanted DNA damage within the human genome.

Methods: Current tools such as BLAST are insufficient for this task and will produce numerous false negatives; furthermore, they cannot account for known polymorphisms in the human genome. This is further complicated by the non-linear nature of the binding penalties associated with gRNA recognition. In order to resolve these issues, we have developed a new database containing all potential cleavage sites within the entire human genome along with all known single nucleotide polymorphisms in dbSNP.

Results: Using a suffix tree, we have indexed the more than 300 million potential cleavage sites across the human genome, which allows for a nearly instantaneous search for potential off target effects across the genome. Tree construction occurs on the order of O(N) ~ Nlog(N) time where N is the size of the database and tree search occurs in O(m) ~ m where m is the length of the query. In practice, the entire human genome and dbSNP were indexed within 72 hours and a query takes less than 10 ms per gRNA.

Conclusions: This database will greatly increase the ability of researchers to design gRNA that can cleave HIV-1 while avoiding off-target effects. Future studies will be performed to validate the off-target predictions using biomedical research laboratory techniques.

PP 9.4

Strategies to overcome active and latent HIV: attack is the best way for defense

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Background: There are two phases of HIV infection in the body, the active phase (the apparent form in plasma viral load) and the latent phase (the hidden form in HIV reservoirs) that contains the genetic code of HIV. They are located mainly in CD4+ T cells (cellular reservoirs) and throughout the body, including the brain, lymphoid tissue, bone marrow, and the genital tract (anatomic reservoirs). They remain invisible to the body immune defenses, and are not sensitive to anti-HIV drugs. Hence, HIV is able to remain a chronic, life-long infection.

Methods: We could overcome HIV via two strategies. The first one is to control the plasma viral load (the apparent form) by three methods: (1) highly active antiretroviral therapy (HAART) that decreases the viral load to undetectable level by preventing HIV from multiplying; (2) Autologous monocyte-derived dendritic cells (MD-DCs) pulsed with heat inactivated autologous HIV-1 that leads to a decrease in viral load after HAART interruption in vaccinated patients with a concomitant increase in HIV-1-specific T cell responses. The vaccine is safe and well tolerated; (3) Heat therapy of human serum. It is possible to inactivate HIV by heat therapy of blood, plasma and other serum samples at 54–56°C for 5 hours. The experiment showed that heat therapy does not alter protein binding. The second strategy is to eradicate the HIV reservoirs (the hidden form). This could be done by two routes. (1) Using the first strategy (HAART) is very slow and not effective because the half-life of the reservoirs is extremely long (44 months). At this rate, eradication of those reservoirs would require over 60 years of treatment with the first strategy. (2) Reactivation of latent HIV reservoirs.

Results: The first strategy turned HIV infection from a death sentence to a manageable disease. As it allows the immune system to stay healthy but it is supposed to be used lifelong; however, this is not an ideal situation for HIV-infected individuals because of drug cost and worries about resistance. We are still in need of new strategies for overcoming HIV such as the second strategy concerned with eradication of reservoirs.

Conclusions: I think that attack is the best way for defense. If we could reactivate the latent reservoirs i.e. convert the HIV from the latent phase (hidden form) to the active phase (the apparent form) that will be recognized by the immune system in continuation of the first strategy to prevent new or spreading infection, we could say that we become very close to eradicate HIV. That reactivation process could be done by stimulating latently infected cells to replicate and express the virus, such cells will die more rapidly (HIV-induced cell death) instead of waiting for decades until they are reactivated.

PP 9.5

Novel CD4-based bi-specific chimeric antigen receptors: toward a functional cure of HIV infection

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Background: Durable control of HIV after cessation of antiretroviral therapy is a much sought-after goal toward a ‘functional cure’ of infection. We are developing a strategy based on targeted killing of HIV-infected cells by genetically modified T cells; when adoptively transferred back to the infected person, these cells will potentially provide the long-term control of infection needed for a functional cure.

Methods: We designed chimeric antigen receptors (CARs) with extremely high potency and breadth, and devoid of potential undesired activities. The CARs contain novel bi-specific extracellular targeting domains composed of sequences from invariant human proteins, and directed against distinct highly conserved determinants on the HIV Env glycoprotein. The targeting domains consist of human CD4 (extracellular domains 1 and 2) linked to the carbohydrate recognition domain (CRD) of a human C-type lectin, which specifically recognizes the high-mannose glycans on gp120. The targeting domains were followed by a transmembrane and intracellular signaling domains of CD28 and CD3 zeta to exert activation and cytolytic functions. To test activity, T cells expressing experimental and control CARs were mixed with HIV-infected autologous PMBC; HIV suppression was assessed by measuring p24.

Results: Compared to a monospecific CD4 CAR, the bi-specific CD4-CRD CARs exhibited extraordinary potency; very similar patterns were observed with genetically diverse HIV-1 isolates. Importantly, the CRD moiety prevented the CD4 component from acting as an entry receptor and rendering transduced CD8+ T cells susceptible to HIV-1 infection.

Conclusion: The minimal immunogenicity predicted for invariant all-human sequences, coupled with likely limits on virus escape imposed by targeting two highly conserved determinants on the Env glycoprotein. The targeting domains consist of human CD4 (extracellular domains 1 and 2) linked to the carbohydrate recognition domain (CRD) of a human C-type lectin, which specifically recognizes the high-mannose glycans on gp120. The targeting domains were followed by a transmembrane and intracellular signaling domains of CD28 and CD3 zeta to exert activation and cytolytic functions. To test activity, T cells expressing experimental and control CARs were mixed with HIV-infected autologous PMBC; HIV suppression was assessed by measuring p24.

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Conclusions: I think that attack is the best way for defense. If we could reactivate the latent reservoirs i.e. convert the HIV from the latent phase (hidden form) to the active phase (the apparent form) that will be recognized by the immune system in continuation of the first strategy to prevent new or spreading infection, we could say that we become very close to eradicate HIV. That reactivation process could be done by stimulating latently infected cells to replicate and express the virus, such cells will die more rapidly (HIV-induced cell death) instead of waiting for decades until they are reactivated.
Results: The R263K substitution was associated with a progressive decline in the levels of integrated HIV DNA.

Conclusions: The R263K resistance substitution impairs the long-term ability of HIV to integrate, possibly decreasing the size of the viral reservoir in individuals who develop resistance against dolutegravir. Therapeutic strategies using dolutegravir to decrease the HIV reservoir should be explored.

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PP 10.0

Induction of HIV from latency by a novel molecule

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Background: Even with the earliest institution of therapy, a pool of long-lived HIV infected cells is established within days of infection and is refractory to ART and the immune response. Compounds that can successfully disrupt this latent pool of virus are critical in cure strategies seeking to reactivate virus while augmenting the immune response as a mean to clear persistent HIV infection. We evaluated the ability of JNJ611, a small peptide shown to lack HDAC inhibitor or mitogen activity, to act as a latency-reversing agent (LRA) in resting CD4 T cells isolated from aviremic participants.

Methods: The ability of JNJ611 to activate the HIV LTR was first evaluated in the J89, 2010 and CI50 Jurkat cell line models of latency encoding the enhanced green fluorescence protein (EGFP) as a marker for LTR activity. EC50 was measured by flow cytometry and toxicity assessed by side/forward scatter gating or Alamar Blue assays. HIV Gag RNA was measured in pools of total or highly purified resting CD4+ T cells isolated from aviremic, durably suppressed participants following overnight exposure to JNJ611. Finally, the ability of the compound to induce replication competent virus from purified resting cells was evaluated in limiting-dilution outgrowth assays using standardized, published methods.

Results: JNJ611 induced a 4–8 fold induction of GFP production in CI50 and J89 cells, but only marginally induced GFP in the 2010 cell line. The compound robustly induced HIV gag RNA expression in the resting CD4+ T cells from three out of four participants and in the total CD4 T-cells from 11 out of 11 participants. However, significant recovery of replication competent virus was only achievable in the resting CD4 T cells from three of six aviremic donors evaluated.

Conclusions: While JNJ611 almost uniformly induced HIV RNA expression, the compound displayed donor to donor variability in its ability to induce outgrowth of replication competent virus. This could reflect interpatient variation in the frequencies of defective genomes (i.e., LRA response and LTR expression without virion production), or the existence of additional obstacles to the production of replication competent HIV following LRA action in the cells of some patients.

PP 10.1

Predicting determinants of long-term HIV control with gene therapy strategies

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Background: Gene therapy to render lymphocytes resistant to HIV infection is a proposed strategy to achieve long-term ART-free remission (e.g. CCR5 modification or delivery of antiviral gene products). Preliminary in vivo studies, however, have had limited success, and there is a need to elucidate the conditions under which modified cells have sufficient selective advantages to reduce target cell density below a critical level required to maintain infection.

Methods: We designed a mathematical model of the competition between wildtype and genetically modified CD4 T cells and the accompanying dynamics of HIV infection. The model was parameterized using data on lymphocyte kinetics, HIV viral dynamics, and the effects of modification.

Results: We find that under a range of scenarios, the most likely outcome is that modified cells co-exist with wild-type cells below the level required to prevent HIV persistence. To control virus off ART, edited CD4+ T cells must have a higher proliferation rate or a longer lifespan even in the absence of virus, or, edited hematopoietic stem cells must be included. The enrichment level of edited cells is highly dependent on the strength of competition between cells for homeostatic proliferation signals. Interestingly, lower thymic contribution to wild-type T cell levels makes invasion easier and engrafment higher. Higher viral fitness can make it easier for edited cells to expand initially but harder to control infection. The potential benefit can be boosted if edited cells are also resistant to certain causes of bystander cell death, or if they provide enhanced immune function.

Conclusions: Modeling suggests that control of HIV using gene therapy is possible only under a narrow range of conditions, and that further measurement and manipulation of immune dynamics during engrafment may be necessary to improve outcomes.

PP 10.2

Neutraplex nanoparticles to target HIV reservoirs

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Background: Monocyte/macrophages are the most important HIV reservoirs outside the bloodstream. Macrophages transport HIV into sequestered anatomically sites such as the lymphoid organs and the brain. Current antiretroviral drugs hardly penetrate into macrophages and anatomic reservoir sites therefore it is highly critical to improve drug delivery in these compartments in order to achieve viral eradication. Nanomedicine is one of the promising approaches to target HIV and enhance drug delivery in viral reservoirs. Here we evaluated the potential of the lipid-based nanocarrier Neutraplex (Nx) for the transport of HIV therapeutics into macrophages and in the brain.

Methods: Cytotoxicity of the Nx nanoparticles (NPs) was assessed using different cell models and various cytotoxicity assays. Cellular uptake of fluorescent Nx NPs was evaluated in THP-1 monocyte-derived macrophages (MDMs) by confocal microscopy. In addition, Nx NPs capability to cross the blood–brain barrier (BBB) was investigated using the immortalized adult rat brain microvascular endothelial cell model (SV-ARBEC) and effect on inflammatory response was evaluated using an in vitro neutrophil apoptosis assay.

Results: Nx NPs showed low cytotoxicity in THP-1 MDMs and were not found to be cytotoxic for HeLa-derived cells (TZM-bl) nor for neuronal cells (Be(2)-M17) up to the highest concentration tested. Confocal studies showed that Nx NPs are rapidly and efficiently taken up by THP-1 MDMs. In addition, Nx NPs were able to transmigrate the endothelial cell monolayer suggesting that they have the capability to deliver drugs through the BBB without affecting neuronal viability. Finally, Nx NPs did not modulate apoptosis of polymorphonuclear neutrophils indicating their low interference with inflammatory response.

Conclusions: Altogether these results indicate that the lipid-based Neutraplex nanocarrier shows potential as a delivery strategy aiming to target HIV in cellular and anatomical viral reservoirs.
**PP 10.3**

Utilizing the binding capacity of CRISPR/Cas9 to target the HIV-1 quasispecies

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**Background:** HIV-1 viral persistence in light of long-term HAART is a major hurdle to a cure. Genomic editing techniques, such as the CRISPR/Cas9 system, hold the promise to permanently excise integrated virus from a host cell. Targets are defined by a 20–25 nucleotide guide RNA (gRNA) molecule complementary to the desired genomic region. However, due to the rapid mutation rate intrinsic to HIV replication, the virus in patients exists as a collection of distinct genomic variants, termed quasispecies. Presented here is a methodology for designing gRNA sequences which will cleave a patient's HIV quasispecies.

**Methods:** PBMC genomic DNA was isolated from patients in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort and the long terminal repeat (LTR) of the HIV-1 quasispecies was sampled using illumina Next Generation Sequencing (NGS). gRNAs were designed using a known positional binding profile to optimally target the quasispecies present in the sample. Using the entire collection of sequences from the CARES cohort, a package of three gRNAs (CARES-3) were designed.

**Results:** The CARES-3 package alone could cleave 70% of the observed quasispecies in the cohort which accounts for >90% of the observable integrated virus of from any patient examined to date. Supplementing this with a package of personalized gRNAs of less than three additional sequences allowed us to target all patients to below our level of detection. Examining longitudinal samples collected over a five year period suggests that the personalized gRNAs will lose their effectiveness if treatment is delayed more than 6 months.

**Conclusions:** Utilizing NGS sampling techniques and computational gRNA design, a package of less than six gRNAs can be developed which will cleave all observable quasispecies within the PBMC compartment. This work presents a step towards understanding the complex task of using excision therapy to target HIV-1 quasispecies in the infected human population.

**PP 10.4**

Effects of heme degradation products on reactivation of latent HIV-1

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We have previously observed that heme argininate, a drug approved for human use in treatment of acute hepatic porphyria, reveals a strong synergism with PKC inducers like TNF-alpha, phospholipid myristate acetate (PMA), prostratin or bryostatin-1 in reactivation of the latent provirus in ACH-2 cells. Heme is physiologically decomposed by action of heme oxygenases into three degradation products: iron (Fe2+), carbon monoxide (CO) and biliverdin that is further converted to bilirubin by biliverdin reductase. Therefore, we have studied the effects of heme degradation products on latent HIV-1 reactivation when added individually to ACH-2 cells harboring integrated HIV-1 provirus or to the A2 clone of Jurkat cells harboring HIV-minivirus expressing EGFP. Addition of iron strongly increased expression of both HIV-1 p24 Ag and EGFP in PMA-stimulated ACH-2 and A2 cells, respectively, as characterized at RNA and protein levels. On the other hand, addition of a CO-donor or bilirubin decreased the PMA-stimulated p24 expression. The reactivation of latent HIV-1 by iron or heme argininate was inhibited by antioxidants N-acetyl cysteine and vitamin E or by an iron chelator desferrioxamine, suggesting that the effects were mediated by an iron-induced redox stress. Finally, we were able to demonstrate the synergistic effects of heme argininate and PMA on HIV reactivation of human peripheral blood mononuclear cells of HIV+ patients cultured ex vivo.

Redox stress was shown to affect epigenetic mechanisms regulating gene expression as well as to activate redox-sensitive transcription factors. Therefore, we propose a model in which heme argininate-mediated redox stress induces chromatin remodeling, affects binding of specific transcription factors to HIV-LTR and allows HIV-1 expression. These results may point towards a new direction in the latent HIV-1 reactivation and therapy.

**PP 10.5**

Genetic variation continues to occur in well-controlled HIV-1-infected patients

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**Background:** The widespread use of antiretroviral therapy (ART) has resulted in effective long-term maintenance with undetectable viral loads. However despite this, we have now clearly demonstrated the continued generation of new mutations during long-term ART.

**Methods:** A set of patients from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort, who have been sampled longitudinally for more than 7 years were studied. Samples for these patients included both pre- and post-ART control of viral replication. Genomic DNA was isolated from samples and the long terminal repeat (LTR) and Tat exon 1 were amplified from proviral sequence. Bayesian Evolutionary Analysis by Sampling Trees (BEAST) phylogenetic trees were built using this sequence information. We modeled shifts in the predominant proviral quasispecies and de novo variation due to mutations combined with selection pressures such as therapeutic interventions, AIDS-defining illnesses, and other factors.

**Results:** This estimated that HIV-1 has an average mutation rate of 5.71/Kb/year, which was reduced by 1.02/Kb/year upon introduction of ART. These results suggest the presence of a low level viral replication in some patients, even in the presence of ART. In a subset of patients, the rate of mutation was not affected by control of clinical parameters due to ART.

**Conclusions:** These studies represent the initial steps in quantifying rates of genetic variation across longitudinally sampled sequences from patients at multiple stages of disease progression. Notably, while long-term therapy reduced estimated mutation rates, they were still non-zero, even in the absence of detectable viral loads. The sequence variation observed may be due in part to differential activation of latent proviral DNA quasispecies and/or low-level viral replication in various reservoirs that occur even in well-controlled patient populations over prolonged time and ultimately detected in the peripheral blood compartment.