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

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

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

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

## PP 1.9

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

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**Background:** DNA methylation is an epigenetic mechanism of HIV-1 latency. The methylation profile of the latent viral 5′LTR is heterogeneous in latency model cell lines and in patient cells in which it increases progressively during cART. Previously, we reported that the DNA methylation inhibitor decitabine (5-aza-2′deoxycytidine) induces different levels of HIV-1 reactivation in latently-infected T cell lines and *ex vivo* patient cell cultures. However, the mechanism of DNA methylation-mediated HIV-1 silencing remains unclear.

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

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

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

## PP 1.10

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

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

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

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

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

## PP 1.11

### Identification of macrophage reservoirs through tropism of HIV-1 envelope

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

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

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

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