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Abstract Book

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Contents

Oral Abstract Session 1

OA1-1

Viral protein U (Vpu) reduces innate sensing of human immunodeficiency virus type 1 (HIV-1)- infected T cells by plasmacytoid dendritic cells (pDCs) via a BST2-dependent process

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Background: BST2 is an interferon (IFN)-induced transmembrane protein that strongly restricts the release of HIV-1 by cross-linking nascent virions on infected cell surface. HIV-1 antagonizes BST2 restriction through Vpu, an accessory protein that downregulates BST2 at the cell surface. Apart from its function as an intrinsic antiviral factor, BST2 has been shown to inhibit TLR7/9-mediated type 1 IFN production in pDCs by engaging ILT7, an inhibitory receptor selectively expressed in these cells. Given that pDC's anti-HIV-1 responses are essentially resulting from TLR7-mediated sensing of infected cells, we examined whether Vpu could modulate detection of HIV-1 infection by pDCs.

Methods: PBMCs or isolated pDCs were co-cultured with T cells infected with wild type (WT) or Vpu-defective (ΔVpu) HIV-1 and innate sensing was evaluated by assessing the levels of IFN released in supernatants 22h later.

Results: Innate sensing of HIV-1-infected MT4 and primary CD4+ T cells by PBMCs was found to be significantly reduced in a Vpu-dependent manner. This effect of Vpu was linked to partial reduction of surface BST2 levels and absence of restricted virions on infected cell surface. Furthermore, downregulation of innate sensing by Vpu was shown to be independent of co-receptor usage, as both R5 and X4 HIV-1-infected cells were sensed with similar reduced efficiency as compared to their ΔVpu counterparts. As previously reported, most of the sensing of HIV-1-infected T cells relied on the presence of pDCs. Interestingly, shRNA-directed depletion of BST2 in HIV-1-infected T cells abrogated the Vpu-mediated downregulation of innate sensing and as such allowed pDCs to sense equally well WT or ΔVpu HIV-1-infected cells. Thus, reduction of innate sensing by Vpu relies on a process that is critically dependent on BST2, excluding the possibility that restricted virion clusters at the surface of ΔVpu HIV-1-infected cells might be sensed more efficiently by pDCs.

Conclusions: Overall, these findings indicate that Vpu-mediated BST2 antagonism allows HIV-1 to dampen-down pDC-mediated sensing and IFN production via a process that likely relies on BST2/ILT7 engagement. Thus, through a highly sophisticated regulation of surface BST2 levels, Vpu appears to promote HIV-1 release while at the same time interfering with pDC antiviral responses.

OA1-2

Dendritic cell-lymphocyte crosstalk stimulates HIV-1 replication and impairs host restriction factor SAMHD1 in dendritic cells

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Background: HIV-1 restriction factor SAMHD1 is counteracted by the viral protein Vpx of HIV-2 or SIV_{sm}/SIV_{mac} but no such viral protein was identified in HIV-1, leading to high restriction of HIV-1 replication in dendritic cells (DCs). Interestingly, we observed a stimulation of HIV-1 replication when DCs were cocultured with primary CD4 T or B lymphocytes suggesting that the HIV-1 restriction in DCs was reduced during DC/lymphocyte crosstalk. The aim of this study was to understand SAMHD1-mediated restriction in DC/lymphocyte coculture.

Methods: Primary monocyte-derived DCs were infected with various R5 HIV-1 primary isolates during 2h, and then cocultured with autologous activated or non-activated CD4 T and B lymphocytes. We distinguished the DCs from lymphocytes by specific membrane staining. After 48h and 72h, the percentages of infected DC-SIGN$^+$ CD3$^-$ MoDCs, CD3$^+$ DC- SIGN$^-$ CD4 T and CD20$^+$ DC-SIGN$^-$ B lymphocytes were determined based on detection of intracellular viral p24 antigen by flow cytometry. Simultaneously, the expression of intracellular SAMHD1 was quantified in DCs. Virus-like particles containing Vpx (VLP-Vpx) and exogenous dNTPs were used as control to decrease SAMHD1 expression and to stimulate HIV-1 replication, respectively.

Results: We found that SAMHD1 expression in DCs was significantly decreased from 80% to 10% when DCs were cocultured with CD4 T or B lymphocytes for 48h and 72h ($p< 0.01$ and $p< 0.05$ respectively, $n\geq6$). Moreover, this decreased expression of SAMHD1 was correlated with an increase HIV-1 replication in cocultured DCs. As controls, VLP-Vpx increased HIV-1 replication and decreased SAMHD1 expression, and addition of exogenous dNTPs to the culture increased HIV-1 replication but without modifying SAMHD1 expression in DCs.

Conclusion: These results demonstrate that CD4 T and B lymphocytes decrease the expression of SAMHD1 in DCs leading to significant HIV-1 replication in these cells. It suggests that HIV-1 restriction factor SAMHD1 could be counteracted by DC/lymphocyte crosstalk and inhibition of this crosstalk would prevent DCs from HIV-1 infection. Therefore, HIV-1 replication and restriction in DCs should be considered in more physiologically relevant models of DC/lymphocyte coculture. This work was supported by EuroNeut41 (FP7- HELTH-2007-A-201038) grant, Dr O. Schwartz kindly provided Ab against human SAMHD1 and VLP-Vpx.

OA1-3

Cell-intrinsic HIV-1 immune responses in conventional dendritic cells from HIV-1 elite controllers

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Background: Recent data suggest that conventional dendritic cells (cDC) are able to mount cell-intrinsic immune responses against HIV-1. However, in the majority of infected individuals, such immune activity is blocked by the host proteins Samhd1 and Trex1 that reduce the accumulation of early HIV-1 replication products. Elite controllers (EC) are able to control HIV-1 replication in the absence of treatment, but immune defense mechanisms in these patients are incompletely understood. Here, we investigated cell-intrinsic immune responses to HIV-1 in cDC from these patients.

Methods: PBMC from EC, untreated chronic progressors (CP), HAART-treated and HIV-1 negative subjects were ex vivo infected with HIV-1. Cellular expression of viral replication products, type I interferons, Samhd1 and Trex1 were analyzed by qPCR.

Results: Paradoxically, we observed the highest susceptibility to HIV-1 infection in cDC from HIV-1 negative persons, while cDC from EC and CP only very weakly supported HIV-1 replication. Yet, reasons for reduced susceptibility to HIV-1 in CP and EC were different: In CP, HIV-1 replication was blocked at the level of early reverse transcription, likely as a result of high-level Samdh1 expression. In EC, reverse transcription was unaltered, and inhibition of HIV replication mostly occurred at the level of viral integration. Functionally, these altered patterns of viral replication dynamics in cDC from EC were associated with increased activation and secretion of type I interferons.

Conclusion: Our data suggest specific alterations of HIV-1 replication patterns in cDC from EC that enable the generation of cell-intrinsic HIV-1 immune responses, while simultaneously blocking productive HIV-1 replication.

OA1-4 LB

Sustained High Levels of Circulating Galectin-9 Despite Viral Suppression Among HIV infected Elite Controllers

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Background: HIV "elite" controllers are typically defined by serial HIV RNA levels below the limit of detection in the absence of anti-retroviral therapy (ART). They represent less than 1% of the HIV population. Despite having limited HIV replication, most controllers have evidence of elevated T cell activation and chronic inflammation, and some exhibit evidence of early atherosclerosis and/or exhibit progressive CD4 T-cell depletion. Chronic exposure (over decades) to viruses like CMV is known to reshape the adaptive immune system. We hypothesized that upregulation of receptors and their ligands known to contain CD8+ T cell activation might be pathogenic in controllers. Galectin-9 is a β-galactoside binding lectin, and functions as an eosinophil chemoattractant and immunomodulator in physiological and pathological setting. Galectin-9 can induce CD4 T cell apoptosis and exerts its immunosuppressive function through engagement with the Tim-3 receptor.

Method: We measured circulating plasma Galectin-9 levels in 20 HIV-infected elite controllers, and 20 demographically-matched HIV-uninfected controls using a Galectin-9 specific ELISA and further assessed the activity of Gal-9 on T cell activity *ex vivo* using a recombinant Galectin-9 protein (rGal-9).

Result: HIV controllers had significantly elevated levels of Galectin-9 compared to uninfected controls (median 263 pg/ml; interquartile range (IQR) 209, 674 versus median 61 pg/ml; IQR 18,143; p< 0.0001). Controllers also had significantly higher frequencies of Tim-3$^+$ CD8$^+$ T cells compared to uninfected controls (median, 9.1%; IQR 6.4,14.18 versus 3.0% IQR 1.58,5.8; p=0.0007). *Ex vivo*, rGal-9 stimulation (5µg/ml) induced IFN-γ release by CD8$^+$ T cells (unstimulated, median 0.05% IQR 0.03,0.12 versus rGal-9, median 0.64% IQR 0.33,1.14; p< 0.05), from peripheral blood mononuclear cells. This was reversed by competitive blockade with α-lactose.

Conclusion: Our data suggests that Gal-9 - Tim-3 cross talk is elevated in controllers, presumably as a consequence of persistent activation of CD8+ T cells. This elevation may be harmful as it could lead to organ specific co-morbidities. Controlling HIV in these individuals with antiretroviral therapy may prevent activation of this pathway, thus avoiding some of the harmful effects of chronic inflammatory states.

OA1-5 LB

Teaching new dogs new tricks: An *in vitro* model of autologous HIV-1 immunotherapy induces CTL from naïve precursors in subjects on ART

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Background: A recent surge in HIV-1 research has focused on developing immunotherapies to eradicate the autologous viral reservoir via cytotoxic T lymphocyte (CTL)-mediated immune responses. To clear infection, new CTL must be primed from naïve precursors, as pre-existing effector memory T cells fail to control viral replication during ART interruption. We have therefore established an *in vitro* model of dendritic cell (DC) immunotherapy for defining efficient priming of naïve CD8$^+$ T cells during ART with engineered DC expressing antigens specific for the autologous HIV-1 reservoir.

Methods: We isolated highly pure populations of naïve and memory CD4$^+$ and CD8$^+$ T cells from HIV-1 infected subjects on ART or from these same subjects prior to HIV-1 seroconversion in the Multicenter AIDS Cohort Study. T cells were stimulated with autologous DC loaded with inactivated HIV-1 derived from the autologous ART reservoir to induce primary CD8$^+$ T cell responses from naïve precursors, or to "re-condition" memory populations. IFN-gamma ELISpot and viral suppression assays were used to evaluate primary CTL effector function against autologous viral antigen.

Results: Mature, type I polarizing DC derived from HIV-1 infected subjects on ART induced primary CTL that suppressed viral replication and survival of autologous virus-infected CD4$^+$ T cells. These primary CTL were specific for the autologous Gag proteome and specifically enhanced the breadth of responses to p17 compared to those found in memory populations. Primary responses during ART did not differ from those induced pre-seroconversion.

Conclusion: We show for the first time that naïve T cells from HIV-1 infected subjects on ART can respond to primary *in vitro* DC vaccination against the autologous virus reservoir and are capable of suppressing viral replication. Thus, chronic, untreated HIV-1 infection did not irreparably impair priming of CTL to autologous virus. These data support the use of DC immunotherapies targeting the autologous reservoir in HIV-1 infected subjects on ART.

Oral Abstract Session 2

OA2-1

Targeting HIV-1 persistence in CD4 T memory stem cells by pharmaceutical inhibition of beta-catenin

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Background: Treatment with antiretroviral combination therapy can effectively suppress active HIV-1 replication, but HIV-1 persists in the human body and rapidly rebounds after discontinuation of therapy. T memory stem cells (Tscm) represent a recently-discovered subpopulation of T cells that persist for extremely long periods of time and are maintained by a stem cell-like developmental program governed through the wnt/beta-catenin pathway. The role of CD4 Tscm for viral persistence is unclear.

Methods: PCR assays were used to determine the amount of cell-associated HIV-1 DNA in sorted CD4 T cell subsets from HAART-treated HIV-1 patients. The presence of replication competent virus within the CD4 T cell subsets was tested using viral reactivation assays. Phylogenetic association studies were performed with viral Env sequences amplified form plasma and individuals CD4 T cell subsets. Effects of the pharmaceutical beta-catenin inhibitor C-82, (the active metabolite of PRI-724, currently tested in clinical trials, e. g. NCT01606579) on CD4 T cell differentiation were studied using ex-vivo culture assays.

Results: HIV-1 DNA in CD4 Tscm from HAART-treated patients were high and exceeded HIV-1 DNA levels in alternative cell subsets. Viral reactivation assays demonstrated that CD4 Tscm harbor replication-competent virus. Viral sequencing studies revealed close phylogenetic associations between circulating plasma HIV-1 strains during early disease stages, and HIV-1 DNA isolated from CD4 Tscm after 6-12 years of therapy, consistent with long-term viral persistence in CD4 Tscm. In vitro culture assays demonstrated that pharmaceutical beta-catenin inhibitors can promote differentiation of CD4 Tscm into more short-lived effector CD4 T cells.

Conclusion: Tscm serve as a long-lasting reservoir for HIV-1 that importantly contributes to viral persistence. Targeting this specific cell compartment by pharmaceutical beta-catenin inhibitors may have an adjunct or additive role for reducing long-term viral persistence in CD4 Tscm.

OA2-2

Longitudinal analysis of infection frequencies and genetic makeup of intracellular HIV-1 from tissue compartments during long-term suppressive therapy

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Background: Efforts to eradicate HIV-1 require a comprehensive examination of the quantity and genetic makeup of HIV-1 populations within infected cells located in tissues throughout the body. Therefore, we conducted a longitudinal analysis of the infection frequencies and genetic makeup of HIV-1 in specific CD4+ T-cell subsets in different tissue compartments from patients on long-term suppressive therapy.

Methods: Using single-genome and single-proviral sequencing techniques, we isolated intracellular HIV-1 genomes derived from defined subsets of T-cells (naïve, central-, transitional-, and effector-memory) from peripheral blood, GALT, and lymph node tissue. Samples were collected at 2 time points (separated by 6 months) from 8 subjects on suppressive therapy (4-12 years): 5 who initiated therapy during acute infection and 3 who initiated therapy during chronic infection. Maximum likelihood phylogenetic trees were constructed using the general time reversible model.

Results: Comparison of the infection frequencies between the 2 time points showed similar (< 5-fold difference) infection rates of memory T-cell subsets from different tissue compartments for most subjects. However, one subject had a 16-fold increase in the infection frequency of peripheral blood effector-memory T-cells at time point 2. Phylogenetic analyses revealed an increase in clonal DNA sequences with no evidence of genetic evolution in this subject. In agreement with findings for time point 1, infection frequencies of all T-cell subsets were higher in subjects treated during chronic infection than acute infection; time point 2 included transitional-memory T-cells which were not examined at time point 1 (6-fold higher infection rate in chronic vs acute; p=0.036). Approximately 30% of the intracellular HIV sequences encoded replication-incompetent virus. In one subject, a clonal species containing a 380bp deletion was dominant, and increased from 71% to 92% over 6 months in peripheral blood effector-memory T-cells.

Conclusions: Our findings suggest the pool of HIV-infected resting memory CD4+ T-cells typically does not change dramatically over 6 months in different tissue compartments, reflecting a relatively stable HIV-infection frequency during suppressive therapy. The increase of clonal HIV-1 sequences from effector-memory T-cells in 2 subjects, especially a large deletion mutant, indicates an expansion of cells with integrated proviral DNA rather than active viral replication.

OA2-3

Identification and characterization of CD4 T cells actively transcribing HIV RNA in peripheral blood

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Background: Determining the phenotype and molecular characteristics of cells within PBMC
that are actively transcribing HIV will be critical to cure strategies. The goal of this study was to identify and characterize cells actively transcribing HIV in peripheral blood.

Methods: Live CD8⁻ PBMCs from 6 HIV-infected individuals not on ART were sorted into CD4 bright, dim, and null populations. Antibodies to markers of T cell activation and HIV antigens were used to further identify cells expressing HIV. Spliced HIV RNAs were identified using primers and probes designed to span tat and rev mRNA splice sites; unspliced HIV RNA was identified using gag primers and probes. The frequency of T cells containing HIV RNA, and the quantity of HIV RNA in each population was determined using limiting dilution RT qPCR. HIV RNA copy number per cell was

determined using values from wells likely to containing a single HIV RNA^{+} cell. Measurable tat and/or rev RNA was used as evidence of active transcription from proviral DNA.

Results: The median frequency of cells containing unspliced RNA in the CD4 bright population was 0.054% significantly greater than the frequency of spliced HIV RNA in the same population, 0.019%. In the CD4 dim population there was routine co-expression of spliced and unspliced RNAs with a median frequency of 0.14%. Median spliced and unspliced RNA copy number increased with decreasing surface CD4 T cell expression. Median unspliced RNA copy number for cells transcribing proviral DNA was 113(20-325) in CD4 bright cells, 198(0-981) in CD4 dim cells and 703(191-2699) copies/cell in CD4 null T cells. Using markers of T cell activation a population of T cells was identified in which 1 in 30 (3.3%) were actively transcribing HIV. Broadly neutralizing env-specific monoclonal antibodies were used to identify HIV-infected cells directly ex vivo.

Conclusion: These data show viral RNA transcription in vivo is associated with strong down regulation of CD4 and identify methods of detecting cells within PBMC with a very high probability of active HIV transcription. Identifying genes expressed by cells actively expressing HIV will allow for the development of strategies to specifically target them for killing.

OA2-4

In Vivo Analysis of the Replication Capacity and Pathogenic Potential of HIV Primary Isolates from an Elite Suppressor

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Background: HIV-infected elite suppressors (ES) have viremia below clinical limits of detection in the absence of anti-retroviral therapy. Host genetics play an important factor in determining viremic control. This control is thought to be mediated by improved recognition of the virus by the immune system and increased genetic pressure. This may yield mutations in the viral genome and have an increased fitness cost and thus lower replication capability. Although isolates from ES have been shown to replicate in vitro studies, their ability to replicate and cause disease in vivo is unknown. Therefore, we sought to evaluate ES-derived isolates in vivo using the BLT humanized mouse model for HIV infection.

Methods: HIV isolates ES38-5 and ES38-9 were cultured from the $CD4^+$ T cells of a previously described ES. BLT humanized mice were generated as previously described. Mice received an intra-venous injection of ES38-5 or ES38-9. HIV viral RNA in the plasma was monitored by real-time PCR. Cell populations were phenotyped using polychromatic flow cytometry.

Results: Both isolates were able to establish sustained HIV replication in vivo. Interestingly, there was an apparent difference between the maximum viral loads seen with the two viruses. This difference cannot be attributed to issues related to the PCR-based detection method or to different donor tissue used for the generation of the humanized mice. In addition, we observed declines of the $CD4^+$ T cells in the blood of the mice infected with ES isolates.

Conclusion: These results demonstrate that the viral isolates from some ES are replication competent and pathogenic as they induce T cell depletion in vivo. Our results support the hypothesis that the low levels of viremia in ES are mediated by the host's genetics and immune response rather than due to a defect in viral replication.

OA2-5 LB

BIT225, a Novel Inhibitor of HIV-1 Release from HIV-1 Reservoirs of the Myeloid Lineage

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Background: Biotron Limited's lead compound, BIT225, blocks Vpu ion channel activity and has anti-HIV-1 activity *in vitro*. The antiviral effect is greater in cells of the monocyte lineage; with circulating monocytes able to differentiate into tissue resident macrophages, a key cellular reservoir of HIV-1. BIT225 is a novel antiviral drug that disrupts viral assembly within the host cell, resulting in a substantial loss of infectivity of the progeny virus. BIT225 was found to be well tolerated in a Phase I clinical trial in healthy volunteers. This study is the first clinical evaluation of BIT225 therapy in HIV-1 infected subjects.

Methods: BIT004 is a phase 1b/2a, placebo-controlled, randomized study of the safety, pharmacokinetics and antiviral activity of BIT225 in 21 HIV-1$^+$, antiretroviral therapy naïve subjects. Subjects received BIT225 (400 mg BID) or placebo treatment for 10 days (randomized 2:1). Twenty-one subjects were enrolled and completed treatment. To explore the potential of BIT225 to reduce the viral burden within the monocyte reservoir, CD14$^+$ monocytes isolated from the peripheral blood on days 0, 5, 10 and 20, were cocultured *ex vivo* with MT4 T cells. *De novo* HIV-1 replication was measured by p24 activity of released virus into the culture supernatant to day 25 of coculture. In addition, monocyte samples were collected for RT-PCR HIV-1 single copy assay analysis.

Results: Cocultures were established with monocytes isolated at days 0, 5, 10 and 20 for both BIT225 treated and placebo controls. Placebo controls demonstrated similar levels of infectious virus released from the monocytes, at all time points, indicative of a stable level of infection. BIT225 treatment resulted in a reduced level of HIV-1 transmission from this compartment. When the BIT225 treated patients were grouped at baseline into those with high versus low viral load (using the median), BIT225 resulted in a significant reduction in the amount of infectious virus released from the monocytes in the higher viral load cohort.

Conclusions: This study's unique design demonstrates that BIT225 can significantly reduce the dissemination of HIV-1 from infected monocytes. Potentially this has important ramifications for diminishing the seeding/re-seeding of the viral reservoir.

OA2-6 LB

Gag-positive reservoir cells are identified by a new technique and are susceptible to HIV-specific cytotoxic T lymphocyte

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Background: Resting CD4+T cells infected with HIV persist in the presence of suppressive anti-viral therapy (ART) and are barriers to a cure. Current belief is that this reservoir persists because it is not expressed. However, the presence of unspliced HIV RNA in resting CD4+ T cells in patients on ART raises the possibility that some expression of HIV Gag may occur.

Methods: We developed a technique to determine if we could detect resting CD4+ T cells that express HIV Gag protein, in HIV infected individuals on ART. To do this, we stained phenotypically resting CD4+ T cells (HLA-DR-, CD25-, CD69-) from HIV infected individuals on ART with an antibody against HIV Gag and sorted for Gag+ and Gag- cells. We then measured HIV DNA in the Gag+ and Gag- fraction to determine if HIV DNA was enriched in the Gag+ population. We also modeled infection of resting CD4+ cells in vitro by super-infecting CD4+ T cells from patients to determine if the super-infected resting CD4+ T cells would be susceptible to CTL lysis after coculture with autologous CD8s.

Results: We found HIV DNA was significantly enriched in the Gag+ fraction in 5 of 5 patients. We calculated based on the fold enrichment that the Gag Positive Reservoir (GPR) represent up to 2% of the resting CD4+ T cells with integrated HIV DNA. When we modeled GPR cells in vitro by superinfecting resting CD4+ T cells from controllers and noncontrollers we found GPR cells did not release infectious virus unless we activated them; however, GPR were susceptible to autologous CTL clearance. Finally, we found the amount of CTL clearance of GPR after CD8 coculture in our in vitro model correlated strongly with the level of integrated HIV DNA in vivo.

Conclusion: A measurable fraction of the reservoir in resting CD4+ T cells expresses HIV Gag proteins in patients on ART and may be subject to CTL clearance. Our data suggest CTL play a role in reservoir size.

OA2-7 LB

HIV-1 DNA levels after antiretroviral therapy in primary infection predict disease progression: the SPARTAC Trial.

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Background: The HIV-1 reservoir is the major barrier to HIV eradication. Measuring the reservoir using molecular qPCR techniques reveals associations with HIV-1 plasma viral load and the likelihood of 'post treatment control'. We investigated whether levels of 'total' and 'integrated' HIV-DNA measured by qPCR after 48 weeks of antiretroviral therapy (ART) were associated with surrogate disease markers and clinical progression in the SPARTAC trial, a large randomized RCT investigating short course ART in Primary HIV Infection (PHI).

Methods: 40 HIV+ve participants with PHI recruited to SPARTAC and randomized to receive 48 weeks of ART were investigated. All participants were recruited from the UK and were male. 38/40 (95%) were infected with subtype B HIV-1. Peripheral Blood Mononuclear Cell (PBMC) samples were taken at enrolment and on stopping 48 weeks of ART. Multi-clade compatible qPCR assays were performed on DNA from CD4 T cell enriched PBMCs to measure 'total' and 'integrated' HIV copies per CD4+ T cell. HIV-1 DNA levels were associated with baseline covariates and times to progression using logistic regression, Kaplan-Meier plots, and Cox models.

Results: Baseline plasma viral load levels and CD4 count were significantly associated with the total ($p=0.0003$ and $p=0.0135$, respectively) and integrated ($p=0.0007$ and $p=0.0161$, respectively) DNA levels after 48 weeks of therapy. There was no association between HIV-1 DNA levels and the estimated time since seroconversion at enrolment. Time to viral load rebound after stopping ART was associated with total ($p=0.027$) but not integrated HIV-1 DNA. Both total and integrated HIV-1 DNA levels were associated with the SPARTAC trial primary endpoint (HR 8.26; $p< 0.0001$ and HR 3.08; $p=0.014$; respectively) which was a composite of reaching either 350 CD4 cells/μl or starting long-term ART. In multivariate cox analyses, total HIV-1 DNA was more strongly associated with clinical progression than other covariates, including plasma viral load at enrolment.

Conclusion: The HIV-1 reservoir level after 48-weeks of treatment strongly predicted disease progression, with total HIV-1 DNA levels being more predictive than integrated HIV-1 DNA levels. These data confirm the significance of the HIV-1 reservoir in circulating CD4 cells and its importance in functional cure strategies.

Oral Abstract Session 3

OA3-1

Activation of the Wnt pathway by natural ligands or small molecule inhibitors activates latent HIV

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Background: Highly Active Antiretroviral therapy (HAART) is very effective in suppressing replication of HIV. However, during HAART, HIV persists lifelong as a latent virus in the patient's memory CD4+T cells. This latent HIV reservoir is transcriptionally competent and cessation of HAART leads to renewed active viral replication. Therefore, in order to eradicate HIV from infected patients, this latent viral reservoir has to be targeted and activated for subsequent elimination by HAART.

Methods: Using J-Lat and S-Lat CD4+ T cell line models as well as ex vivo infected primary CD4+ T cell models reflecting HIV latency we examined the role of the Wnt signaling pathway in regulation of transcription of the latent HIV LTR. We also used various biochemical assays probing the nucleosomal landscape of the latent and Wnt-activated HIV LTR to delineate the mechanism by which Wnt signaling regulates HIV.

Results: We find that activation of the Wnt pathway by natural ligands or small molecule inhibitors resulted in activation of the latent HIV LTR. Treatment of latently HIV infected cells with activators of the Wnt pathway resulted in recruitment of TCF/LEF and b-catenin, the molecular effectors of Wnt signaling, to the latent HIV LTR. Wnt-mediated activation of the latent HIV LTR was synergistically enhanced in the presence of histone deacetylase inhibitors, a class of drugs currently under clinical investigation for activation of latent HIV.

Conclusions: Targeting the Wnt pathway by small molecules and Wnt agonists may be an attractive strategy in a combinatorial therapy aimed at activation of latent HIV infected cells followed by their elimination in the presence of HAART.

OA3-2

Inducing HIV-1 in latently infected T cells with an autologous full length HIV-1 representing the majority intrapatient virus population

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Background: Complete eradication of HIV with antiretroviral drugs is almost insurmountable, as the virus persists in cellular reservoirs as latent proviral integrants. Most approaches to induce the latent HIV-1 pool involve some type of cell activation through mitogens, cytokines/chemokines, or HDAC inhibitors to up-regulate gene expression, which by default may also activate HIV-1 mRNA expression from latent proviruses. Prior to treatment, HIV-1 primarily infects HIV-specific CD4+ T cells that then transitions to the latently infected memory T cell population. Thus, we propose that the most effective and specific activator of latently infected T cells is the patient´s HIV-1 quasipecies prior to treatment.

Methods: The entire HIV-1 genome and population was amplified from multiple plasma samples prior to HAART. The near full length (nfl) HIV-1 genome was RT-PCR amplified as a full fragment, in overlapping halves or thirds are recombined into a yeast-based vector via homologous recombination/gap repair. Through a positive/negative selection system, yeast colonies are grown as population for subsequent proviral plasmid purification and transfection into 293T cells to produce a replication incompetent vector based on the intrapatient HIV-1 population prior to treatment.

Results: The autologous HIV-1 vector, a NL4-3-based vector, and a cocktail of flu/tetanus/CMV antigens were loaded onto the patient DCs and then co-cultured with isolated T cells. DCs and T cells were obtained from patients after 3 years on stable HAART. In three different patients, the autologous HIV-1, present by DCs, induced at least 30-fold higher HIV-1 production from the T cells than did the NL4-3 and 100-fold higher than the Flu/TT/CMV cocktail. In contrast, gamma interferon ELISPOTS on the DC-antigen-T cell cocultivations revealed 10- to 100-times more spot forming units with the Flu/TT/CMV antigen cocktail than with the autologous HIV-1 vector.

Conclusion: These findings suggest that the entire intrapatient HIV-1 population in a safe, replication incompetent vector may be the best and most specific stimulus to drive HIV-1 out of the latent T cell pool. We suspect that most latently infected T cells are also HIV- specific in antigen recognition and that during active virus replication the majority of the acquired immune response was focused on HIV-1.

OA3-3

Destruction of the residual active HIV-1 reservoir by Env-specific immunotoxin

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Background: HIV reservoirs are responsible for HIV persistence in patients during antiretroviral therapy (ART). Persistence of HIV infection despite ART is marked by two phenomena - the persistence of quiescent but replication-competent provirus and the persistent production of HIV RNAs by an undefined population(s) of cells. This work demonstrates the efficacy of a novel targeted cell killing approach to deplete productively infected cells in vivo.

Methods: Humanized BLT mice were constructed, HIV infected, and treated with ART essentially as we have previously described (Denton et al. J. Virol 2012) or not treated with ART. Pharmacokinetic analysis and determinations of latently infected cells were performed as previously described (Choudhary et al. J. Virol 2010, 2012). An immunotoxin targeting HIV Env (3B3-PE38)(Bera et al. Mol Med, 1998) or vehicle was administered every other day for 14 days to ART treated animals. Animals were harvested and levels of residual RNA in tissues were compared between control, ART treated and ART + 3B3-PE38 treated mice using a combined mixed effect statistical model. In addition, individual infected cells were quantified using in situ hybridization and compared between experimental arms using the Mann-Whitney test.

Results: We examined HIV reservoirs in BLT humanized mice during ART and performed a tissue-specific pharmacodynamic analysis that reveals the effect of antiretrovirals on systemic HIV-1 RNA levels at the individual tissue level. Despite strong systemic HIV suppression in plasma (p< 0.001), HIV expression in tissues continued to persist. To eliminate HIV producing cells, we augmented ART with an immunotoxin targeting HIV Env (3B3-PE38) and found that addition of the immunotoxin further reduced RNA levels up to 3.2 logs in individual tissues (p< 0.001). This result was confirmed by in situ hybridization. This significant reduction in cell-associated HIV RNA production over ART alone highlights the susceptibility of the residual active HIV reservoir to targeted cytotoxic therapy.

Conclusion: Our in vivo data show that complementing ART with an immunotoxin targeting HIV-producing cells leads to a dramatic further reduction in the levels of productively infected cells in all tissues analyzed. These results suggest an effective strategy to eradicate HIV with a combinatorial approach.

OA3-4 LB

Entinostat is a histone deacetylase (HDAC) inhibitor selective for class 1 HDACs and activates HIV production from latently infected primary T-cells

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Background: Combination antiretroviral therapy (cART) is unable to eradicate HIV due to the persistence of latently infected resting T-cells. One approach to eliminate latency is to stimulate virus production from latently infected cells using compounds such as histone deacetylase inhibitors (HDACi). Recent clinical trials of the HDACi vorinostat in HIV-infected patients on cART confirmed that vorinostat can activate virus transcription in resting CD4+ T-cells in vivo. Evaluation of newer HDACi is critical to identify more potent, less toxic and more selective compounds. In this study, we aimed to determine the relative potency and toxicity of a panel of HDACi in clinical development in latently infected cell lines and a primary T-cell model of latency. In addition, we sought to demonstrate which HDACs were expressed and were critical for maintenance of latency in resting CD4 T-cells.

Methods: Latently infected CCL19-treated CD4+ T-cells and latently infected cell lines ACH2 and J-Lat were treated with a panel of HDACi including entinostat, vorinostat, panonbinostat and MCT3. Viral production and cell viability were compared. Expression of cellular HDACs was measured by western blot and PCR. Association of HDACs with the HIV long terminal repeat (LTR) using latently infected CCL19-treated primary CD4+ T-cells in the presence and absence of specific HDACi was determined by chromatin immunoprecipitation (ChIP).

Results: We demonstrated considerable variation in the potency and toxicity of HDACi in latently infected primary CD4 T-cells and cell lines. All HDACi tested activated HIV production in latently infected primary T-cells with greatest potency demonstrated with entinostat and vorinostat and greatest toxicity with panobinostat. Following addition of HDACi in vitro, there were no changes in markers of T-cell activation or expression of the HIV co-receptors CXCR4 or CCR5. ChIP analysis of latently infected CCL19-treated primary CD4 T-cells showed binding by HDAC1, 2 and 3 to the LTR with removal of HDAC1 and 2 following treatment with the HDACi vorinostat and HDAC1 only following treatment with entinostat.

Conclusion: The HDACi entinostat, selective for inhibition of class I HDACs, induced virus expression in latently infected primary CD4 T-cells making this compound an attractive novel option for future clinical trials.

Oral Abstract Session 4

Impact of 12 months HAART on cell-associated HIV-DNA in acute primary HIV-1 infection in the OPTIPRIM-ANRS 147 trial

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Background: Early Initiation of a potent HAART at the time of primary HIV-1 infection (PHI) could block the virological and immunological storm and limit the establishment of HIV reservoirs. The randomized multicenter OPTIPRIM-ANRS147 trial was designed to evaluate the impact of a 24-month HAART initiated at the time of PHI on cell-associated HIV-DNA.

Methods: Inclusion criteria included HIV-1 western-blot with ≤ 4 antibodies, positive HIV-RNA and CD4 < 500/mm^3 if PHI was asymptomatic. Patients were randomized 1:1 to receive darunavir/r, emtricitabine/tenofovir (Arm 1) or darunavir/r, emtricitabine/tenofovir (Arm 2). The primary endpoint was the between-arm difference in cell-associated HIV-DNA decrease at M24. Clinical evaluation, cART tolerability, CD4 count, HIV-RNA and HIV-DNA were collected during the trial. Hereinafter, we present the overall results of HIV-RNA and HIV-DNA decrease at 12 months. The 24 month-treatment period will be ended in July 2013.

Results: A total of 90 patients (median age: 35.5 years) were enrolled from May 2010 to July 2011, the median time from estimated date of infection was 35 days and 43% had HIV1 Western-Blot with ≤1 antibody; 92% were male and 96% had symptoms. At baseline median values for CD4, HIV-RNA and HIV-DNA were 472 cells/mm^3 [IQR: 368-640], 5.4 log copies/ml [IQR: 4.9-5.8], and 3.65 log copies/10^6 PBMC [IQR: 3.35-4.02], respectively. Treatment was well tolerated with only 2 serious adverse effects (1 lipodystrophy, 1 acute pancreatitis), both in Arm 2. The CD4 difference at M12 was +239 cells/mm^3. Plasma HIV- RNA decrease was >2 log cp/ml in 86 % subjects at M1 and HIV-RNA was < 50 cp/ml in 47%, 84%, 91% at M3, M6, and M12, respectively. Cell-

associated HIV-DNA median [IQR] decrease from baseline was -0.75 [-0.94, -0.52], -1.12 [-1.33, -0.79] and -1.37 [-1.64, -1.15] log copies/10^6 PBMC at M3 (n=73), M6 (n=63), M12 (n=39), respectively; 51.3% subjects had HIV-DNA level ≤2.3 log copies/10^6 PBMC at M12.

Conclusion: This is the first trial showing such a rapid and intense decrease in cell-associated HIV-DNA within one year. This probably results from initiation of HAART very early after HIV infection.

OA4-2

In chronically HIV-1-infected patients long-term antiretroviral therapy initiated above 500 CD4/mm^3 achieves better HIV-1 reservoirs' depletion and T cell count restoration

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Background: Our group has already shown that antiretroviral therapy (ART) initiated during primary HIV-infection is associated with a profound reduction of viral reservoirs and restores optimal immunity. Present study aimed whether such benefit could be also observed in chronically infected patients (Fiebig VI) treated before the CD4+ count falls below 500/mm^3 (CHI$_{>500}$).

Methods: Prospective cohort-study of HIV-1-infected patients enrolled from 2005 to 2012
in Orleans' Hospital. Patients on ART achieving plasma viral load (PVL) < 50 copies/ml were included and followed as long as PVL remained controlled. Patients were stratified according to CD4+ nadir: >500, 350-500, 200-350 and < 200 cells/mm^3. Total HIV-DNA in PBMC was measured at least once a year (Biocentric, Bandol, France), before (when possible) and during treatment; T-cell count (CD4+, CD8+) and PVL were measured every 3-4 months. Factors leading to a low reservoir in blood (HIV-DNA < 2.3 log/M PBMC) and a normal T-cell count (CD4+ >500/mm^3, CD4/CD8 >1) were determined using Cox proportional-hazards regression.

Results: 283 patients were included (n=28, 26, 113 and 116 for >500, 350-500, 200-350 and < 200 strata, respectively) and followed during a median 4 years. At last visit, CHI$_{>500}$ patients had significantly lower HIV-DNA level (median=2.50 log) as compared with other strata (2.88, 2.78 and 2.91, respectively; p=0.003). Immune reconstitution was faster and better in CHI$_{>500}$ than in other strata (median CD4+=883/mm^3 vs. 722, 645 and 520, respectively; p< 0.0001; median CD4/CD8=1.22 vs. 0.96, 0.85 and 0.68, respectively; p<0.0001). In multivariate analysis, ART started above 500/mm^3 was highly predictive to achieving low HIV-DNA (< 2.3 log) and normal T-cell count (HR=32.4, 95%CI:10.5-99.5, p< 0.0001) (Fig.1).

[Figure 1]

Figure 1: Kaplan-Meier estimates of the probabiliy of achieving a low level of HIV-DNA together with a normal T cell count according to CD4+ nedir stratification
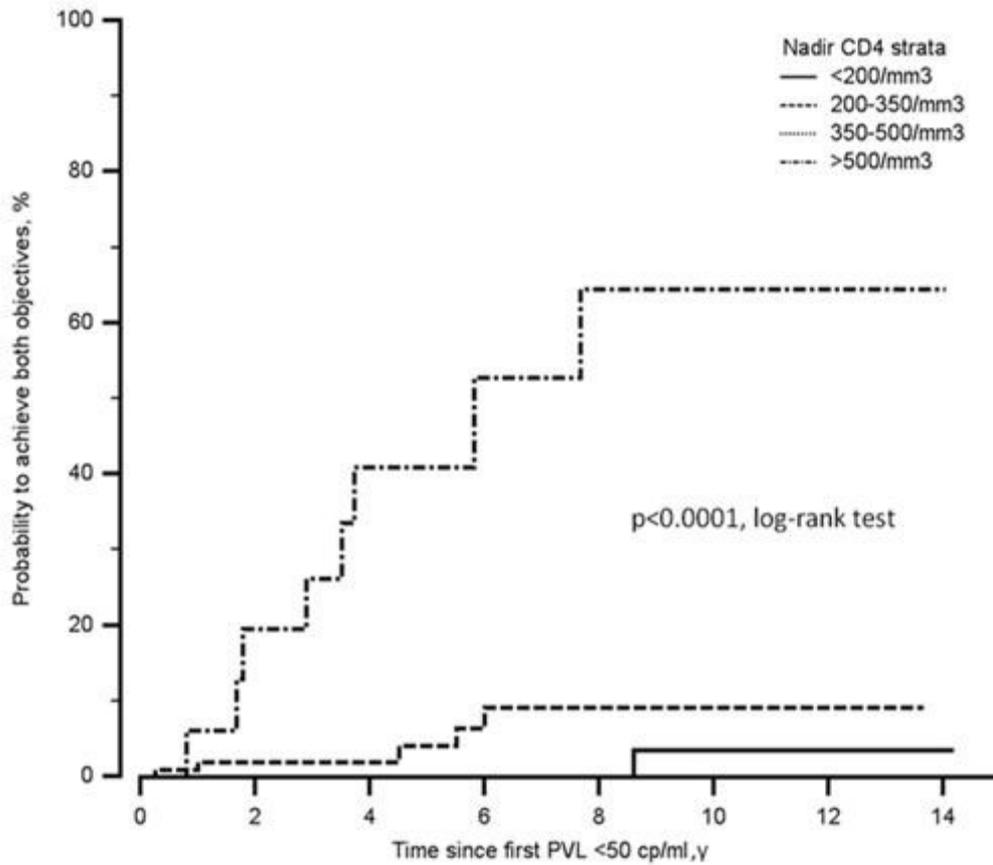
Conclusion: Even patients treated at the chronic phase could benefit from early treatment. This reinforces the value of public health programs to promote early HIV testing and treatment, especially among highly-exposed risk groups.

OA4-3 LB

Passive transfer of neutralizing monoclonal antibody KD-247 reduces plasma viral load in patients chronically infected with HIV-1：A Phase-1b clinical study of a humanized monoclonal antibody KD-247 (KD-1002).

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Background: KD-247 is a humanized monoclonal antibody (mAb) with potent neutralizing activity. The epitope recognized by the mAb was mapped to IGPGRA of the V3-tip that covers about a half of subtype B. The objectives of this Phase 1b study were to evaluate the safety and tolerability of 3 infusions of KD-247 over 2 weeks in HIV-1 seropositive individuals, to determine the pharmacokinetic (PK) parameters when administered as above and to assess the effect of KD-247 infusions on plasma HIV-1RNA load and on CD4$^+$ T cell counts.

Methods: A Phase 1, double-blinded, placebo-controlled, dose escalation cohort study of KD-247 in asymptomatic HIV-1 seropositive individuals who did not currently need antiretroviral therapy. Eligible subjects were randomized to 1 of 3 cohorts to receive 3 infusions of 4, 8, or 16 mg/kg of KD-247 or placebo over a 2-week period. A minimum of 6 active-agent subjects and 3 placebo subjects for each dose cohort were to complete 2 weeks of infusions. Dose escalation proceeded only after safety data through Day 18 for all subjects in the lower-dose cohort were reviewed.

Results: A total of 30 subjects were enrolled in the study with 20 receiving KD-247 and 10 subjects receiving placebo. KD-247 was safe and well tolerated and we observed moderate but significant decreases in HIV-RNA in the 8 and 16 mg/kg cohorts of KD-247. We observed two in six cases of 16 mg/kg cohort that achieved >1 log reduction of HIV-RNA and long-term suppression of viral load for one patient despite significant decrease in plasma concentration of the antibodies, suggesting that effects other than neutralization or loss of fitness of the virus with the mutations acquired. Two subjects in the 16 mg/kg cohort had selections and/or mutations in the V3-tip region that suggested neutralization escape. No tropism shifts was observed for these mutants.

Conclusion: Results should be interpreted with caution due to the small sample size. However, these results taken together, suggest that neutralizing mAbs would be a promising candidate of intensification therapy added-on to the current suppressive cART aiming toward functional cure of the disease.

OA4-4 LB

In Depth Investigation of Peripheral and Gut HIV-1 Reservoirs, HIV-Specific Cellular Immunity, and Host Microchimerism following Allogeneic Hematopoetic Stem Cell Transplantation

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Background: We previously reported the loss of detectable peripheral blood HIV-1 reservoirs in 2 individuals following reduced-intensity conditioning allogeneic hematopoetic stem cell transplantation (RIC-alloHSCT) from wild-type CCR5 donors. To understand further the impact of alloHSCT on viral reservoirs, we studied the longitudinal effects of HSCT on host microchimerism and HIV-specific cellular immunity, and tested rectal tissue and peripheral blood mononuclear cells (PBMCs) obtained by leukapheresis for evidence of residual HIV-1 DNA or replication-competent proviruses up to 4.3 years post-transplantation.

Methods: The following experiments were performed: 1) collection of PBMCs by leukapheresis for large-scale HIV-1 quantification of genomic DNA and viral co-culture from purified CD4+ T lymphocytes (assays using 5 million PBMCs were repeated up to 30 times for each patient), 2) HIV-1 DNA PCR on rectal tissue (one patient), and 3) microchimerism studies of residual donor PBMCs. We also investigated HIV-specific cellular immune function by ELISpot IFN-gamma screenings of total PBMCs involving comprehensive HLA-specific peptide panels on the above patients in addition to a third RIC-alloHSCT patient that died 6 months post-transplantation from recurrent lymphoma; a fourth patient who received an autologous HSCT served as a control.

Results: No HIV-1 DNA was detected from PBMCs from both previously-reported RIC-alloHSCT patients indicating at least a 3 to 4 $\log_{10}$ decrease in peripheral viral reservoir size post-transplantation. No HIV-1 p24 antigen was detected by viral co-culture from purified CD4+ T cells, and no HIV-1 DNA was detected in rectal tissue. Residual host cells constituted less than 0.001% of PBMCs post-HSCT and may have represented circulating non-hematopoietic cells. No HLA-specific or pooled HIV-1 peptides elicited a strong HIV-specific immune response from all patients either before or after allogeneic or autologous HSCT.

Conclusion: HIV-1 remained undetectable from peripheral blood and rectal tissue after RIC-alloHSCT in patients on ART despite the testing of very large numbers of PBMCs or CD4+ T cells. The lack of detectable HIV-1 was in the setting of full donor chimerism and weak HIV-specific cellular immunity. Analytical treatment interruption remains the definitive experiment to test the full impact of RIC-alloHSCT on HIV-1 persistence.

Poster Exhibition

A5 – Entry (attachment, receptors and co-receptors, penetration and tropism)

PE2

HIV-1 entry and *trans*-infection in astrocytes: implications for cure and eradication

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Background: HIV-1 penetrates the central nervous system (CNS) during early infection, establishing a viral reservoir in macrophages and astrocytes. We recently demonstrated that astrocytes are extensively infected and may represent a significant HIV-1 reservoir within the CNS. Whilst direct infection of astrocytes maybe a long-term reservoir, short-term astrocyte reservoirs may exist by storing, protecting and concentrating cell free virus. This latter concept is presently referred to as *trans*-infection, where a cell can bind and harbor virus to be transferred to a recipient. Here, we characterised HIV-1 entry and *trans*-infection of astrocytes. Elucidating both is essential to understanding the HIV-1 CNS reservoir, and for development of eradication strategies.

Methods: Astrocytes were pulsed with non-saturating amounts of HIV-1 BaL and virus half-life determined by p24 ELISA or co-cultured with different ratios of JLTRG cells (T-cell line expressing LTR-EGFP) and *trans*-infection determined by FACS analysis of EGFP in the T-cells. Astrocytes were infected with an EGFP content-labelled HIV-1 YU2$_{ciGFP}$ and immunofluorescently stained for endosomal markers (CD63/CD81/CD107/EEA1) to characterise the compartment harboring virus. Endosomal compartments were modified by using inhibitors (dynasore, dyngo-4a) or by shRNA silencing of CD81.

Results: Astrocytes bind and harbor virus in the short-term, with virus detectable out to 72 hours and an initial half-life of 1.2 hours. Astrocytes can transfer HIV-1 to JLTRG cells with similar efficiencies observed for 1:1 and 1:10 astrocyte:JLTRG ratios. The virus-containing compartment required 37°C to form and was trypsin-resistant. HIV-1 and CD81 demonstrated clear co-localization, while CD63, CD107b and EEA1 did not. SVG-lowCD81 cells were generated using shRNA silencing of CD81 (42% reduction). Experiments with SVG-lowCD81 revealed no loss of co-localization between CD81 and HIV-1, despite reduced CD81 levels.

Conclusion: The CD81 compartment observed herein, has been shown elsewhere (in other cell types) to be a relatively protective compartment. Within astrocytes, this compartment may be actively involved in virus entry and/or spread. The ability of astrocytes to transfer virus, without *de novo* viral synthesis, suggests they sequester and protect virus and thus facilitate not only viral dissemination in the CNS but add further complexities to HIV eradication strategies within this compartment.

A8 – Regulation of viral gene expression and replication

PE3

Suppression of HIV-1C virus production in human PBMCs by dsRNA induced Chromatin remodeling of LTR promoter

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Background:
HIV-1 subtype C is highly infectious clade which claims approximately 50% of worldwide HIV-1 infection and predominates in sub-Saharan Africa, China, India and other regions of Asia, has been less studied in comparison to subtype B which accounts for 10% worldwide infection. The 5'LTR of HIV-1 acts as the promoter and regulates the transcription of all downstream viral genes. So, dsRNA mediated transcriptional gene silencing (TGS) of HIV-1C viral genes would be a good approach to suppress viral production. In our study, we have targeted the HIV-1C LTR promoter using dsRNA to induce TGS and decrease viral gene transcription.

Methods:
siHa cells stably expressing luciferase gene under the HIV-1C LTR promoter was used in the presence of Tat protein for the screening of potent siRNA which can induce TGS. Luciferase expression (mRNA) and activity was measured by Real-Time PCR and dual luciferase assay respectively. The chromatin modification at the targeted region after siRNA transfection was studied using CHART-PCR and ChIP assay. HIV-1C infected human PBMCs were transfected with siRNA and Gag-p24 antigen level was determined by ELISA.

Results:
Multiple siRNAs targeting the enhancer and core region of HIV-1C 5'LTR promoter were screened and of these, one siRNA showed significant decrease in luciferase activity and its mRNA expression post transfection. The CHART-PCR and ChIP assay showed that this siRNA mediated TGS was caused by methylation of histone tails like H3k9me2 and H3K27me3, which leads to heterochromatization of the targeted LTR region. This siRNA mediated TGS also causes the suppression of viral replication and marked reduction in apoptosis of HIV-1C infected human peripheral blood mononuclear cells (PBMCs).

Conclusion:
We have identified a potent siRNA which causes the heterochromatization of HIV-1C 5'LTR promoter, leading to suppression of viral gene expression and its productive infection for at least 21 days after a single transfection in *ex-vivo* experiments. TGS of HIV-1C causes a long-lasting decrease in viral replication because it operates through heritable epigenetic modification.Thus dsRNA mediated TGS can be used as a therapeutic modality for HIV-1C infection in future.

A9 – Cellular factors necessary for HIV replication

Restriction of HIV-1 infection in dendritic cells avoids induction of IFN-α-mediated responses and reduces chemokine expression. Impact on viral escape and establishment of CD4 reservoirs

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Background:
Both immature (IDC) and mature (MDC) dendritic cells display strong restriction to productive HIV1 infection state but are capable of presenting the virus to T cells thereby enhancing the infection of lymphocytes and the spread of virus in the body. The restriction to infection can be overcome by the presence of Vpx protein of HIV2. In this work we analyze the regulation of gene expression in DC infected by HIV-1 in conditions of restrictive (-Vpx) or productive (+Vpx) replication and differences in gene expression between productively infected IDC and MDC.

Methods:
IDC were generated from blood monocytes treated with GM-CSF and IL4. Final differentiation induced with LPS. DCs were infected with NL4.3-Δenv-GFP viral particles VSV-pseudotyped. Vpx was incorporated into virions by co-transfection of a Vpx-expressing plasmid in producer cells. RNA was isolated, labeled and hybridized to a Whole Human Genome Microarray (Agilent). Expression ratios (log2) of mRNA from IDC and MDC were calculated using non-infected cells values as baseline. Only probes with q-value < 5% and 2 fold change were considered as statistically significant.

Results:
The frequency of productive infection increased from 30 to 75% in IDC and from 5 to 20% in MDC with Vpx-virions. Gene expression showed that HIV-1 infection induced interferon (IFN)-responsive gene expression in IDC and down-regulated chemokine genes in MDC.

Major gene expression changes in productively infected by HIV-1			
IDC: Up-regulated molecules	**Fold**	**MDC: Down-regulated molecules**	**Fold**
IFI27	3.85	CXCL10	-4.56
IFI44L	3.6	APOBEC3A	-3.16
IFI6	3.02	CXCL11	-2.94
TCHH	2.59	CXCL9	-2.82
IFIT1	2.46	MYH7	-2.61

[Gene Expression Changes]

Conclusion:
Productive HIV-1 replication in IDC triggers antiviral responses through the induction of IFN-related-genes. Down-regulation of chemokines in MDC cells could potentially prevent transmission of HIV to lymphocytes. Thus, paradoxically, restriction of HIV-1 replication in DC would results in viral escape from IFN-a response and increased transmission to CD4 lymphocytes in the immune synapse, thus increasing the size of CD4 reservoirs.

PE5

Involvement of Human Topoisomerase II isoforms in the Strand transfer events of HIV-1 Reverse Transcription

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Background: HIV-1 reverse transcription (RTn) involves synthesis of double strand DNA (ds DNA) from viral genomic RNA. RTn is promoted by a coordinated activity of viral and host proteins through unknown mechanism. Topoisomerase II (Topo II) alpha and beta maintains topological reorganization of dsDNA regions and catalytic inhibition of these isoforms inhibited viral replicative cycle. The aim of this study is to understand the role of Topo II isoforms in HIV-1 early replication.

Principal findings: Topo IIα and β showed differential expression during early hours of HIV-1 infection where Topo IIα expression decreased during the first hour and then increases after 4 h, while Topo IIβ showed relatively higher expression at 1 and 4 h. Thus both α and β isoforms promote early events of viral replication. In Topo IIα and/or β down regulated cells, transcription of viral genes *gag, pol* and *env* as well as proviral DNA synthesis was abolished, affecting HIV-1 replication. Molecular analysis of RTn events showed that strong stop DNA synthesis was unaffected in Topo IIα and/or β down regulated cells, while other downstream events of RTn such as first strand transfer, full length minus strand synthesis, and second strand transfer were completely inhibited. Further, these results were confirmed by strong association of Topo IIα and β with HIV-1 reverse transcriptase in cellular localization studies.

Conclusion: These results suggest a potential role of Topo II isoforms in HIV-1 RTn through regulating the downstream events of strong stop DNA synthesis during RTn possibly by promoting strand transfer events and alignment of RNA-DNA hybrids.

A10 – Cellular and tissue reservoirs

PE6

Efficient infection of healthy donors´ monocyte derived macrophages by erythrocyte-associated HIV

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Background: Erythrocytes of HIV+ individuals present p24 Antigen, RNA-HIV and bounded-specific antibodies. This HIV- erythrocyte association was observed to be mediated by immune complexes or complement factors with CR1 receptors in the erythrocyte membrane, and/or the virus associated to the Duffy antigen receptor for chemokines (DARC).
Taking into account the importance of macrophagotropic strains in HIV infection, and the close contact of erythrocytes and macrophages during the erythrocyte-clearance process in the spleen and liver, this work focuses on the capacity of the erythrocyte-associated HIV to infect macrophages.

Methods: Erythrocytes and monocyte derived macrophages (MDM) were obtained from 12 healthy donors' buffy coats. Experiments were carried out with erythrocytes and MDM from the same donor. Erythrocytes were incubated with virus in 3 different conditions: **A-** macrophagotropic strain BaL virus; **B-** BaL virus + complement (normal human serum); **C-** BaL virus + antibodies (pool of inactivated serum of HIV+ patients with VL< 50 copies/ml) + complement. After quantification of p24 Ag on erythrocytes, MDM were incubated with erythrocytes obtained in conditions **A**, **B** and **C** for 2 hours at 37ºC. At 2 to 14 days post inoculation (pi), p24Ag was determined on the culture supernatant and immunofluorescence was performed on MDM.

Results: The amount of virus captured by erythrocytes in the 3 *in-vitro* conditions was similar to that present in erythrocytes of patients.
Under condition **B** (HIV + complement) p24Ag was positive in MDM culture supernatant in all samples (80 pg/ml at day 13 pi). Besides, in 4 samples p24Ag was positive in the culture supernatant in conditions **A** and **C**, indicating a productive infection of the MDM. Infectivity was proved on Ghost cells and in other MDM. The MDM immunofluorescence for p24Ag was positive for all samples as from day 6 pi, even in those where no supernatant p24Ag was found (A and C), suggesting that HIV-1 can enter MDM, although no viral replication was observed.

Conclusion: Given that erythrocyte-associated HIV through complement maintains its infectivity on MDM, this viral fraction could be important in the early stages of infection and for reservoir generation.

PE7

Erythrocyte-associated HIV in a model of HIV infection

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Background: Recently, binding of HIV to erythrocyte membrane has been demonstrated in infected patients, including those efficiently treated with HAART. During physiological clearance of immune complexes, erythrocytes are in close contact with spleen and liver macrophages. HIV can take advantage of this process and infect macrophages, which may act as viral reservoirs.
Commonly used mathematical models for HIV replication do not consider the erythrocyte-associated HIV fraction. This study was aim to measure different parameters in this fraction and include them in such models. This can be useful for patients with undetectable viral load undergoing simplified maintenance therapies and those included in attempting viral eradication protocols.

Methods: To analyze the kinetics of attachment/ detachment of HIV to erythrocytes, the erythrocytes of 6 healthy donors were incubated with HxB2 virus at different times. After incubation, p24Ag was measured, and the amount of bound virus was calculated by the difference between the incubated virus and that measured in the erythrocyte supernatant.
Extensions of Perelson's mathematical models were performed in order to include the erythrocyte-associated HIV subpopulation and two kinds of susceptible cells, T cells and macrophages.

Results: Attachment/detachment studies showed that the greater proportion of virus binding occurs in the first 5 minutes and most of it stays attached for hours on the erythrocyte membrane. Infectivity of erythrocyte-associated HIV fraction is well conserved, better than in plasma (data not shown).
The model predicts 1) the association with erythrocytes favors virus macrophagotropic variants in detriment of lymphotropic ones. 2) taking into account the assumption that viruses that emerge from macrophages in spleen and liver can bind preferentially to erythrocytes, viral replication on macrophages will be detected on erythrocytes earlier than in plasma (studies in our laboratory have shown that in patients on HAART blips are associated with virus on erythrocytes).

Conclusion: When the erythrocyte-associated HIV fraction is included in mathematical models of HIV infection, the results vary and the predictions agree with observations in patients, such as detection of virus on erythrocytes even during effective HAART. Therefore, this fraction should be considered when the kinetics of virus replication is studied.

Differential impact of APOBEC3-driven mutagenesis on HIV evolution in diverse anatomical compartments

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Background: Previous studies on HIV quasispecies have revealed HIV compartimentalization in various tissues within an infected individual. Such HIV variation is a result of a combination of factors including high replication and mutation rates, recombination and APOBEC3-host selective pressure. So far, little data is available on the impact of APOBEC3-induced Guanosine-to-Adenosine (G-to-A) mutations on viral compartimentalization.

Methods: To evaluate the differential impact of APOBEC3-editing in HIV-1 compartments, we studied the level of G-to-A hypermutation in HIV-1 protease and reverse transcriptase bulk sequences among 30 patients for whom peripheral blood mononuclear cells (PBMCs) and body tissues or fluids were collected on the same day (14 paired PBMCs/Cerebral spinal fluid (CSF); 8 paired PBMCs/renal tissues; 8 paired PBMCs/rectal tissues). Differences in the G-to-A mutation frequencies were analyzed using the Hypermut 2.0 program.

Results: APOBEC3-mediated hypermutation were identified in 35% (11/30) of subjects in at least one viral reservoir. Hypermutated sequences were observed more frequently in viral sanctuaries (Total n=10; CSF, n=6; renal tissue, n=1; rectal tissue n=3) compared with peripheral blood (Total n=5). Accordingly, APOBEC3 editing generated more G-to-A drug-resistance mutations in sanctuaries: 3 patients' CSF (i.e G73S in protease; M184I, M230I in RT) and 2 other patients' rectal tissues (M184I, M230I in RT) while such mutations were absent from paired PBMCs. Conversely, in one patient, hypermutation was observed in PBMCs sequences (including M184I in RT) while not detected in rectal tissue sequences.

Conclusion: APOBEC3-induced mutations observed in peripheral blood may underestimate the overall proportion of hypermutated viruses in the body as these mutations were observed more frequently in sanctuaries compared to PBMCs in our study. This phenomenon reinforces the role of APOBEC3 editing in HIV compartimentalization *in vivo*. The resulting mutations may favor escape to antiretrovirals in these compartments in conjunction with a lower penetration of drugs in some sanctuaries. On the other side, because hypermutated sequences often harbor inactivating mutations, this study suggests that accumulation of defective viruses may be more dominant in sanctuaries than in peripheral blood of patients on effective HAART.

PE9

Factors associated with a low HIV reservoir in patients with prolonged suppressive antiretroviral therapy

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Background: The relevance of a low level HIV reservoir in patients with prolonged therapy is not well understood. Our objective is to determine factors that influence the establishment of small reservoirs in long-term treated patients (excluding treatment since acute infection) to a level similar to HIV Elite controllers (< 100 HIV Total DNA copies/10^6PBMCs).

Methods: Cross-sectional study involving patients receiving highly active antiretroviral therapy (HAART) with plasma HIV RNA< 50 copies/ml for whom total DNA measurement were performed. Patients treated since early acute infection or receiving cancer chemotherapeutic/ immunosupressive agents were excluded from the study.

Results: Overall, 246 patients receiving HAART with undetectable viremia were involved in the study. The median HIV DNA was 372 copies/10^6PBMCs. Fifty eight patients had a low HIV DNA level< 100 copies/10^6 PBMCs. A low proviral DNA was associated with an ultrasensitive Viral Load< 1copy/mL (p< 0.0001), a lower HIV RNA zenith (p< 0.0001), a higher CD4 T cells nadir (p=0.023), lower current CD8 T cells counts (p=0.010) and a higher current CD4/CD8 ratio (p=0.003). In addition, such a low reservoir was also associated with a higher time spent with undetectable HIV-1 RNA (p=0.018). Other factors such as length of time on HAART and duration of HIV-1 infection were not associated with levels of HIV-1 DNA.

Conclusions: The obtention of a low HIV DNA level, reflecting a limited pool of infected cells is associated with a high CD4 nadir and a low HIV RNA zenith, reinforcing the need to institute antiretroviral treatment early during chronic infection to control HIV reservoir. In addition, the fact that low reservoirs are associated with a higher time spent with undetectable viremia is consistent with previous findings showing that the pool of infected cells decreases with time under HAART. Our results reinforce the idea that a small reservoir may be related to stronger control of residual viremia that in turn keeps the immune activation system to a low activation status.
This study helps to define factors associated with low proviral DNA setpoints after long-term treatment and should be useful to identify future candidates for strategies aiming at eradicating HIV.

PE10

T cell activation positively correlates with cell-associated HIV-DNA level in PBMCs in viremic patients with acute or chronic HIV-1 infection

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Objective: To evaluate the relationship between levels of T-cell activation and HIV-DNA in PBMCs in viremic patients with acute or with chronic HIV infection before and after antiretroviral treatment (ART) interruption (TI).

Methods: Patients with chronic infection (CHI) included in a substudy of the ANRS 116 SALTO trial, a multicenter study of TI that enrolled patients who started ART with CD4 count >350 /mL and VL < 50,000 copies/ml and exhibiting at TI (baseline) CD4 counts >450/mL and VL < 400 copies/ml were selected for the study and monitored at BL and M12 of TI. Patients diagnosed with primary HIV infection (PHI) were also investigated before introduction of cART. CD4 and CD8 T cell activation were analyzed in relation with HIV-DNA level in PBMCs using Spearman tests.

Results: In ART-treated CHI patients (n=25), at baseline, median (IQR) level of HIV-DNA was 2.56 log copies/10^6PBMCs (2.00; 2.93) while at 12 months of TI, median (IQR) HIV-RNA and HIV-DNA levels were 4.25 (3.69; 4.57) and 3.13 (2.67; 3.49), respectively. At baseline, there was no relationship between HIV-DNA levels and T-cell activation, whether assessed by expression of HLA-DR and/or CD38 on CD4 CD8 T cells. In contrast, at M12 of TI, HIV-DNA levels strongly correlated with the proportion of CD8 and CD4 T cells expressing CD38 (r=0.77, p< 10^{-3} and r= 0.72, p< 10^{-3}, respectively). In untreated PHI patients (n=22), plasma HIV-RNA and HIV-DNA levels were 5.7 (4.8; 6.1) and 3.7 (3.0; 4.0), respectively. Again, HIV-DNA levels correlated with the proportion of CD8 expressing Ki-67 (r= 071; p< 10^{-3}), CD38 (r= 0.64, p=0.001) and co-expressing HLA-DR and CD38 (r= 0.47; p=0.034). Moreover, HIV-DNA levels also correlated with the proportion of HLA-DR+CD4 T cells (r= 0.53; p= 0.013) and Ki67+CD4 T cells (r= 0.61; p= 0.003).

Conclusions: Levels of T-cell activation positively correlate with HIV-DNA levels in viremic patients with acute or chronic infection. The lack of association between HIV-DNA levels and T-cell activation in ART-treated patients suggests that the residual immune activation is not directly dependent on the size of the latent HIV reservoir at least in early ART-treated patients.

A11 – Mechanisms of HIV persistence

PE12

Evaluation of residual HIV-1 replication among individuals receiving different antiretroviral treatment regimens

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Background: Residual HIV-1 replication among individuals under antiretroviral therapy is an obstacle towards reduction of chronic HIV related micro-inflammation.

Objectives: To determine the levels of residual viral replication of HIV-1 in distinct subgroups of patients inferred by quantification of episomal HIV DNA, quantification of total HIV DNA and quantification of HIV-1 specific antibodies.

Methods: 109 patients were divided into 5 groups: first suppressive therapy with efavirenz (26), first suppressive therapy with boosted protease inhibitors (PI) (25), salvage therapy using boosted PI (27), salvage therapy with raltegravir (15) and virological failure (16). Quantification of episomal and total DNA was performed by real-time PCR. Specific antibody quantification was performed using enzyme immunoassay capture.

Results: Episomal DNA amplification was positive in 36 out of 109 patients' samples (33%) and quantification of total DNA was obtained in 94 patients' samples (86.3%). Individuals on salvage therapy using Raltegravir presented lower prevalence and lower quantitation of epissomal DNA as compared to other treatment groups (p=0.03). There was no differences between groups in quantification of total DNA (p=0.298) or antibodies (p=0.126). The HIV-1 proviral load was higher among individuals with positive epissomal DNA (p=0.01). There was a negative correlation between the episomal DNA quantification and *(i)* duration of treatment with undetectable viral load, *(ii)* CD4 counts, and *(iii)* CD8 counts. There was a higher prevalence of episomal DNA and a higher quantification of total DNA in virologic failure group (p=0.009 and 0.06 respectively). The antibody quantitation was higher among individuals on initial treatment using efavirenz compared to initial treatment with PIs (p=0.027).

Conclusions: Duration of treatment, CD4, CD8 counts, and raltegravir based regimens relate to decreased residual viral replication (epissomal DNA). The relationship between episomal DNA and total DNA suggests replenishment of proviral reservoir with potential impact on HIV persistence. Lower antibodies levels among patients with PI based initial treatment may suggest a more effective HIV suppression of these regimens, with higher capacity of decreasing the HIV antigenic component.

A13 – Strategies to target and eradicate reservoirs

PE14

Establishment of macaque CD4+ T cells and CD34+ hematopoietic stem cells resistant to SIV infection using Zinc Finger Nucleases technology

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Background: CCR5 is the major HIV co-receptor, and individuals homozygous for a 32-bp deletion in Ccr5 gene are resistant to infection by CCR5-tropic HIV-1. Therefore, the CCR5 co-receptor provides a unique opportunity to exploit gene knockout technologies for anti-HIV therapy. The Berlin patient highlights the potential therapeutic benefit of CCR5 disruption in treatment and possible eradication of HIV infection.

Methods: Various gene therapy approaches to block CCR5 expression are currently being evaluated. The targeted cell populations include both mature peripheral T cells and Hematopoietic Stem Cells (HSC). The loss of CCR5 in HSC appears to have no adverse effects on hematopoiesis. We are pursuing the use of engineered Zinc Finger Nucleases (ZFNs) to permanently disrupt the CCR5 open-reading frame. In our system, the ZFN set chosen generates a DNA double-strand break in the region encoding for the second extracellular loop of CCR5, thus mimicking a CCR5delta32 mutation. CCR5-targeted ZFNs are evaluated *in vitro* in our laboratory, targeting mature CD4+ T cells and hematopoietic stem cells isolated from naïve-uninfected macaque blood, bone marrow and umbilical cord samples.

Results: We engineered SIV-resistant macaque CD4+ T cells using CCR5-ZFNs. After nucleofection of mRNAs encoding for ZFNs into CD4+ cells isolated from macaques, we show that these cells were resistant to in vitro SIVmac239, SIVmac251, and SIVagm infections as shown by the absence of p27 expression. We then focused on the modification of HSC isolated from macaque femoral bone marrow and umbilical cords. We established conditions required to purify and grow macaque CD34+ HSCs in vitro to maximize the efficiency of CCR5 gene disruption while minimizing any adverse effects on cell viability or hematopoietic potential. We successfully engineered CCR5-modified macaque hematopoietic stem cells that were resistant to SIVmac239 infection after in vitro differentiation and expansion on thymocytes.

Conclusion: We demonstrated the feasibility of using ZFN technology to establish CD4+ T cells and hematopoietic stem cells resistant to SIV infection in macaque. The generation of a nonhuman primate model using this modern molecular-based strategy might significantly help in the design of new therapies to prevent viral infection and eradicate HIV infection in human.

PE15

Dual approach to HIV-1 cure: Activation of latency and restoration of exhausted virus-specific T cell function

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Background: The persistence of HIV-1 infection in patients suppressed by combination antiretroviral therapy (cART) is due to the presence of latent reservoir(s), including that within resting CD4+ T cells. An additional factor in persistence is the dysfunction of HIV-1-specific T-cells in infected individuals. Thus, elimination of the latent HIV-1 reservoir may require a therapeutic strategy that incorporates a combination of activation of latent HIV-1 virus as well as restoration of HIV-specific T-cell responses.

Methods: We have developed multiple assays for identification of compounds that reactivate latent HIV-1. These assays involve an integrated and quiescent HIV-1 LTR-reporter present in both primary T cells and immortalized T cell lines. A parallel screening approach was employed.

The ability of immunomodulatory therapies to affect human chronic viral infections also is being investigated with *in vitro* assays, in animal models and in clinical trials. As proof of concept, nivolumab (anti-PD-1; BMS-936558) has been tested in the context of chronic HCV in human subjects.

Results: Triage of the hits from these screens has revealed compounds capable of activating latent HIV-1 reporters and virus in multiple contexts.

Although this trial did not meet its primary endpoints, a minority of subjects experienced a significant virologic response.

Conclusion: We hypothesize that a combination of approaches will be necessary for reduction of the HIV-1 reservoir leading to eradication of latent virus or functional cure. To this end, we have developed a dual approach to discover agents with complementary mechanisms of action and whose combination may eradicate latent HIV-1 infection. Future studies will be directed at a combination of these approaches.

PE16

Combination of proteasome and Hsp90 inhibitors induces autophagic cell death in HIV-1-infected macrophages and CD4+ T cells: approaches to elimination of the virus reservoirs

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Background: CD4+ T cells and macrophages are major in vivo HIV-1 reservoirs where the virus replicates and persists. Development of approaches to better pharmacological targeting these reservoirs is of importance for anti-AIDS therapy. In our previous studies, we have revealed the sensitivity of the HIV-1-infected cells to various inhibitors of proteasomes or heat shock protein 90 (Hsp90). The present research work was aimed at examining effects of two pharmacological inhibitors of proteasomes (bortezomib) and Hsp90 (17AAG) on HIV-1-infected and non-infected CD4+ T cells and macrophages from AIDS patients.

Methods: The pools of CD4+ T cells and monocytes were isolated from 14 HIV-1-seropositive patients with established AIDS. Sorting of the HIV-1-infected and non-infected cells was performed on a FACS using labels to cell surface markers. The harvested monocytes were in vitro developed into adherent macrophages. The separated subpopulations of CD4+ T cells and macrophages were treated with bortezomib (a proteasome inhibitor) and/or 17AAG (an Hsp90 inhibitor). Apoptosis/necrosis was determined with FITC-annexinV/propidium iodide staining. The autophagic cell death was monitored on lethal autofagosome-autolysosome transition.

Results: It was found that co-treatment with 100-400 nM bortezomib and 10-250 nM 17AAG induces autophagy in both the HIV-1-infected macrophages and CD4+ T cells what was manifested in massive formation of autophagosomes and subsequent appearance of large autolysosomes. Afterwards 60-75% of those cells underwent autophagic death (i.e. lethal autolysis) with total elimination of the infectious matter. The observed effect on HIV-1-infected macrophages was similar to what is exerted by vitamin D. The minor fraction (25-30%) of the infected and drug-treated macrophages and CD4+ T cells died via necrosis or apoptosis without clear signs of autophagy. Importantly, non-infected macrophages and CD4+ T cells exhibited no autophagy and only low (15-20%) apoptosis following the same drug co-treatment, while 80-85% of them remained viable.

Conclusion: We conclude that HIV-1-infected macrophages and CD4+ T cells are much more sensitive to the co-treatment with inhibitors of proteasomal and Hsp90 activities as compared with the non-infected cells. Simultaneous administration of such inhibitors (e.g. bortezomib + 17AAG) may be used for beneficial clearance of the AIDS-patients' organism from the HIV-1 reservoirs.

PE17 LB

Maraviroc (MVC) intensification can activate NFkB through CCR5 and the expression of its target genes in resting CD4^{+}-T-cells in suppressed HIV-1-infected patients

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Background: Activation of CCR5 intracellular signaling pathways leading to transcription factors activation could promote HIV-1 transcription in resting CD4^{+}-T-cells. In previous experiments, we showed the activation of NFkB and specific target genes in resting CD4^{+}-T-cells cells from naïve patients with HIV-1 RNA>1,000 copies/mL who received 10 days of maraviroc (MVC) monotherapy in a clinical trial (TROPISMVC, NCT01060618). The present clinical trial aims to explore if MVC could trigger this effect in suppressed HIV-1-infected patients.

Methods: MARAVITRANS (Eudra CT: 2012-003215-66) is a clinical trial of 10 days MVC intensification. Blood samples were drawn at baseline, after 10 days of MVC and 18 days after MVC withdrawal. From 10 patients, activated and resting CD4^{+}-T-cells were separated by magnetic beads coupled to monoclonal antibodies (MACS® Technology) and aliquots of 5 million cells were frozen. NFkB and NFAT activity were detected by an ELISA-based kit consisting of plates coated with oligonucleotides mimicking consensus binding sites specific for each transcription factor (TransAM[TM] NFkB family and TransAM[TM] NFATc1, Actif Motif), following the manufacturer's instructions. NFkB activity was estimated measuring target genes' expression by real-time PCR of the extracted RNA.

Results: NFkB activity was detected in resting CD4^{+}-T-cells in 6/10 patients; results expressed in fold change (FC) compared to baseline. The presence of MVC increased NFkB activity in resting CD4^{+}-T-cells, as summarized in the following table.

| Patient | NF-κB Activity (FC) | | | |
| | Activated CD4+-T-cells | | Resting CD4+-T-cells | |
	Day 10 (on MVC)	Day 28 (off MVC)	Day 10 (on MVC)	Day 28 (off MVC)
1	2.32	1.8	0.88	1.07
2	0.3	0.4	0.4	0.5
3	1.4	1.4	0.5	0.7
4	0.8	1.3	1.3	0.9
5	0.5	15.4	3.5	20.1
6	0.5	1.3	4.3	2.4
7	1	0.7	1.8	1
8	1	0.1	2.4	2.2
9	1.17	0.8	1.8	1
10	1.45	0.95	1.36	1.29

[Table 1]

Upregulation of at least one NFkB targeted gene (IFN-g, IL-6, IL-10, TNF-a) was observed in cases where NFkB activity was detected. In case of NFAT, no significant activity was documented.

Conclusion: Our results suggest that MVC activates NFkB, and the subsequent expression of targeted genes, in resting CD4^{+}-T-cells from suppressed HIV-1-infected patients, as previously observed in treatment-naïve ones. Through this pathway, MVC could trigger HIV-1 transcription in resting cells thus accelerating the decay of the HIV-1 cell reservoir.

PE18

Metal Nanoparticles Reduce Intracellular HIV-1 Replication and Stimulate Growth of HIV-1 Infected PBMC

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Background: Noble nanoparticles have the potential to be used as therapeutic agents in HIV-1 infection. Noble nanoparticles have higher electron resonance potential than organic drug molecules to arrest HIV and enhance cell growth. Silver nanoparticles have been shown to exhibit promising cytoprotective activities towards HIV-infected T-cells and arrest HIV by blocking gp120-CD4 interaction. Metal nanoparticles are engineered as non toxic and employed as it have unique signal propagation properties. Combined nanoparticles may arrest HIV replication by functionalize with variety of molecules and produce specific signals and proliferate PBMC by inducing cytokines. The aim is to examine the effect of combined nanoparticles on growth and viral replication in HIV-1 infected human PBMC.

Methods: Combinations of Silver, Tin and Zinc (AgSnZn) nanoparticles were processed as nanomedicine (NM), 28nm in size(less than 25nm is toxic) to treat HIV-1 infected cells in cell culture. PBMC cells were isolated from HIV-1 positive laboratory sample and cultured with RPMI 1640 medium, IL-2, PHA and 10%FCS in 96 well plates at the cell density of 2.5×10^5 cells/ml with NM and without NM for 96 hrs. The Immuno Peroxidase Test (IPT), an intracellular viral antigen detection assay to assess intracellular antigen and cell quantification assay (CQA) to quantify cells were employed in both cultures to find intracellular HIV and to assess cell growth. Experiments were repeated ten times.

Results: The cells quantity in NM treated cultures had more population (10×10^5 cells/ml) than the non treated cells (4.5×10^5 cells/ml),which was 55% ($p<0.0001$,t-test of ten assays) higher shows that NM has induced cell growth. IPT revealed absence of HIV (no golden granules) in cytoplasm of NM treated cells but present in non NM treated cells which indicate the NM has arrested the intracellular HIV ($p<0.001$,Chi-Squired test of ten assays).This results show that the NM can arrest HIV and improve cell count.

Conclusion: This study has revealed that the NM arrests the intracellular HIV and proliferate PBMC as well. This NM could be considered for therapeutic approach to HIV-1 infected individuals. Further studies are to be carried out to characterize the molecular mechanisms of the effects.

PE19

HIV Resistance to Dolutegravir (DTG) Simultaneously Diminishes Viral DNA Integration into Host Cells and Viral Replication Fitness: Implications for HIV Reservoirs

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Background: No HIV-infected patient, naive to the therapeutic use of integrase strand transfer inhibitors (INSTIs), has yet developed resistance against dolutegravir (DTG). To characterize the resistance profile of DTG, we selected for resistance in tissue culture against this compound.

Methods: We grew HIV-1 of different subtypes in both MT-2 cells and in peripheral blood mononuclear cells over protracted periods, with the concentration of DTG being incrementally increased from 0.05 nM, i.e. 4 times less than the EC50. After a total of 6 months, a final drug concentration of 50-100nM was achieved, beyond which virus could no longer be grown. Viral DNA was sequenced and the biological relevance of any mutations was confirmed biochemically and by site-specific mutagenesis.

Results: R263K or G118R followed by H51Y were the most frequent integrase resistance mutations to arise in subtypes B and C, respectively. R263K alone conferred an approximate 2-5-fold level of resistance to DTG and a 30% drop in levels of recombinant integrase strand transfer activity and viral replicative capacity. Although H51Y alone did not significantly affect either enzyme activity or DTG resistance, the combination of R263K together with H51Y increased DTG resistance to about 12-fold accompanied by a ≈70% loss in each of viral replication capacity, the ability of viral DNA to become integrated into host cell DNA, and integrase strand tranfer activity as measured in biochemical assays using purified integrase enzyme. Over > 1 year, no additional possibly compensatory mutations were identified.

Conclusion: These results stand in contrast to those obtained with other drugs, whereby secondary mutations increase overall levels of drug resistance and simultaneously increase viral replication and enzyme function, and help to explain why primary resistance to DTG has not yet arisen in clinical studies. Validation of these findings in animal models may support the use of DTG in strategies aimed at purging HIV cellular reservoirs, perhaps over several cycles of DTG treatment, if it can indeed be shown that resistance to DTG is not compensated by other mutations located either within the integrase gene or elsewhere in the HIV genome.

A14 – Mucosal transmission

PE20

The intestinal microenvironment affects the susceptibility of DCs to HIV-1

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Background: The intestinal mucosa is the major site of HIV-1 entry and persistence. We previously showed that intestinal colonic dendritic cells (DC) are actively recruited to extend cellular projections across an intact epithelial barrier in response to CCR5-using virus. DCs, being thus a first target for HIV-1 during transmission, may also act as reservoir. However the susceptibility of intestinal DC to HIV infection in the intestinal microenvironment has been poorly investigated, due to difficulties in isolating mucosal DC.

Methods: Myeloid DC (mDC) obtained from the intestinal colonic lamina propria and from blood were identified as Lin (CD3, CD14, CD16, CD56, Cd19)-HLADR+ CD11c+ cells and further characterized for the expression of HIV-1 receptors.Supernatant obtained from an ex vivo culture of healthy human colonic mucosa was used to condition monocyte-derived DC in an in vitro model as to mimic the exposure of DC to intestinal microenvironment. Conditioned-DC (C-DC) were analyzed by flow cytometry for the expression of HIV-1 receptors and activation markers, and incubated in vitro with either R5 or X4 HIV-1 to study their susceptibility to infection.

Results: C-DC displayed a significant down-regulation of CCR5 and CD4, an up-regulation of CXCR4 and a moderate modulation of DC-SIGN expression compared to unconditioned DC. Intestinal conditioning did not induce activation of the cells. Interestingly, both R5 and X4 HIV-1replicated less efficiently in C-DC compared to unconditioned DC. Among several cytokines and chemokines analyzed, colonic supernatants contained the CCR5-binding chemokines Mip1b and MCP-1, whereas the CXCR4 ligand SDF-1a was absent. IL-10 and IL-2, described to induce CXCR4 up-regulation on DC, were also detected. Thus, this specific intestinal milieu may determine the observed phenotype.Of note, colonic mDC showed lower CCR5 and higher CXCR4 expression compared to blood derived mDC, and a similar activation profile, which confirmed the results obtained after intestinal conditioning.

Conclusion: The intestinal microenvironment can condition the phenotype of DCs by modulating the expression of the HIV receptors and in turn also the susceptibility to and replication of the virus. Our model is relevant to study the role of mucosal DC in HIV-1 infection, spreading and persistence in the intestinal mucosa.

A19 – Intrinsic cellular defences and restriction factors

PE22

A Population-based Matched-cohort Study on Insertion/Deletion Polymorphism of the *APOBEC3B* gene and Risk of HIV-1

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Background: The human APOBEC3 (A3) family proteins (A3A-H) potently inhibit replication of HIV-1. In human populations, a high-frequency distribution of the *A3B* deletion genotype is observed. Previously, several groups have reported controversial observations on the effect of *A3B* gene deletion on HIV-1 acquisition and disease progression. Here, in order to verify the effects of A3B on HIV-1 infection *in vivo*, we investigated the insertion (I)/ deletion (D) polymorphism frequencies of the *A3B* gene in a matched cohort in Japan.

Methods: The *A3B* genotype was analyzed by PCR with genomic DNA from blood or buccal membrane. The effect of *A3B* gene deletion on disease progression was evaluated by comparing acquisition of Syphilis, HBV and HCV, as well as $CD4^+$ T cell counts and viral load before starting cART among three genotype groups; deletion-homozygous (D/D), hemizygous (D/I), and no deletion (I/I) genotypes. Susceptibility to HIV-1 infection was assessed based on the frequencies of three genotypes between the infected and uninfected cohorts. HIV-1 replication kinetics and the infectivity were assayed *in vitro* by using healthy donor $CD4^+$ T cells. Fischer exact and Mann-Whitney U tests were used for statistics.

Results: 228 HIV-1-infected patients of Japanese men who have sex with men (MSM) and 207 uninfected Japanese MSM were enrolled. Our *A3B* genotyping analysis showed no significant difference in the ratio of *A3B* genotype between the infected (D/D 8.3%, D/I 44.7%, and I/I 46.9%) and the uninfected (D/D 8.7%, D/I 39.6%, and I/I 51.7%) cohorts (p=.55). In addition, the parameters of disease progression resulted in quite similar frequencies among the three genotype groups. These results suggest no significant effect of *A3B* gene polymorphism on the HIV-1 transmission or the disease progression. Furthermore, the *in vitro* kinetics of HIV-1 replication and the infectivities of the virus in $CD4^+$ T cells were comparable between D/D and I/I (p=.31 and p=.86, respectively).

Conclusion: Our analysis of a population-based matched cohort showed that loss of *A3B* gene is not associated with the risk of HIV-1 susceptibility and disease progression. The results suggest *in vivo*, A3B could play an unknown role,but not in eliminating HIV-1.

PE23

Increased levels of APOBEC3 and interferon mRNA in PBMC of Highly Exposed Seronegatives Individuals (HESN)

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Background: Innate immune response plays an important defense against different viruses including HIV. Variation in their expression and function could contribute to risk of HIV infection. Type I interferons are induced by a variety of viruses elements or by their genetic sequences through the TLR or RIG pathway. Interferons activate cellular restriction factors such as APOBEC3 (A3) and TRIM5 proteins. Some reports showed significantly increased APOBEC3G (A3G) mRNA in PBMCs from HESN, and stimulated PBMCs of long-term non-progressors (LNTP). The question remains whether celular restriction factors expression is the result of exposure to HIV or its gene products. To address this question, we measured gene expression of interferon, TLR and RIG pathways in PBMC, monocytes, LB, CD4 and CD8 T cells of Mexican ES and Controllers (CT).

Methods: The study sample consisted of 20 subjects. 5 healthy controls (HC), 5 HESN, 5 CT and 5 HIV infected patients without treatment with >300 CD4+ (PT). Total RNA was extracted from sorted monocytes, LB, CD4 and CD8 T cells of unstimulated and stimulated PBMC either with interferon alfa or TLR's agonists. cDNA quantification of 96 genes including APOBEC proteins and TRIM5 was performed by real time PCR based on Nanofluid technology (Biomark, Fluidigm), relative quantification was done by duplicate. Statistical differences between groups were assessed with Mann-Whitney U test.

Results: There were significantly increased levels in relative quantification of A3A, A3F and A3G mRNA in unstimulated PBMC from HESN compared with HC ($p< 0.05$), and PT ($p< 0.01$). Similarly, interferon-inducible genes like IFI16, IFI44, INFA1, INFA2, INFA4 e INFB1 mRNA levels in unstimulated and interferon stimulated PBMC from HESN were significantly increased compared to HC ($p< 0.05$), and PT ($p< 0.01$). We found no significant difference in mRNA levels of both APOBEC and interferon molecules between CT compared with HC.

Conclusion: Our data suggest that HESN present increased innate immune response mediated by IFNs. Their expression can be transcriptionally up-regulated by type I IFN through ISRE/IRF-E responsive elements in their promoters. Upregulation of these factors might increase the activity of restriction factors that inhibit or block viruses such as retroviruses, including HIV-1.

A20 – IFN-I (viral inhibition, immunomodulatory functions)

PE24

Characterization of HIV-1 Gag and Nef T cell Responses in an HIV-1 infected Kenyan Population

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Background: The development of an efficacious vaccine against HIV-1 is one of the most pressing challenges facing modern medicine. The identification of the optimal and dominant HIV-1 specific responses is important for defining immunogenicity in HIV-1 vaccine trials. We aimed to characterize and quantitate nature of HIV-1 specific T cell responses to the Gag and Nef proteins in a Kenyan population, where the dominant circulating virus is subtype A.

Methods: The immunodominant T cell responses in 50 HIV-1 infected individuals were screened by IFN-g ELISpot assay using Group M consensus Gag and Nef peptides (15 mer overlapping 11 aa) arranged in protein-specific and matrix pools and confirmed at the single peptide level. The CD4 counts ranged from 350-1461cells/µl, with a median of 522 cells/ µl in antiretroviral treatment naive HIV-1 infected Kenyan population.

Results: PBMCS from 80% and 58% of the study population had responses to Gag and Nef antigens, respectively. The Gag p24 subunit dominated the magnitude and breadth of T cell immune responses, followed by the p17 subunit.There was a wide range in the magnitude of responses observed among the responders for HIV-1 Group M consensus Gag (100-2050 SFC/10^6cells) and Nef proteins (100-1720 SFC/10^6cells).

Conclusion: The identification of Gag and Nef-specific T cell responses targeting epitopes from multiple immunodominant regions in an HIV-1-infected Kenyan population may provide useful insights into the design of new immunotherapies and vaccines for effective control of HIV-1 infection.

A21 – NK cells and dendritic cells

PE25

Analysis of NK cell receptor-ligand expression in the prevention of AIDS-defining opportunistic infections in patients with low CD4+ T cell count

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Background: CD4+ T lymphocyte cell count is commonly used in the management of HIV infection. However, HIV infected individuals with low CD4+ cell counts may have dramatically different clinical evolutions, ranging from asymptomatic infection to AIDS. In this study, we investigated NK cell populations and the expression of NK cell ligands on CD4+ T cells in HIV-infected patients presenting with low CD4+ but different clinical features (AIDS *versus* non-AIDS).

Methods: We recruited 19 HIV-infected patients with a history of comparable CD4+< 200/ml at presentation (11 AIDS and 8 non-AIDS at diagnosis) and 10 healthy subjects (HD). AIDS diagnosis was PCP in 9 cases and neurotoxoplasmosis in 2 patients. The mean duration of the antiretroviral treatment and CD4+ at sampling was similar in both groups. Multiparametric flow cytometry and specific mAbs were used to evaluate phenotypic and functional parameters of NK and T cells, including activating and inhibitory receptors, CD69, HLA-DR, CD107a and NK ligand expression by CD4+ (PVR, nectin-2 and MIC-A) either before or after *in vitro* activation. Statistical analysis included U-test and Spearman's test for correlation.

Results: CD4+ immune reconstitution was comparable among AIDS and non-AIDS patients. Expression of NKp46 as well as its density on CD56+ NK cells was significantly higher in non-AIDS when compared with AIDS ($p < 0.05$). A similar finding was observed also for the expression of NKp30. AIDS and non-AIDS patients had a substantial increase of CD69 expression on CD56+ NK cells ($p < 0.05$) and HLA-DR both on CD56+ NK ($p < 0.05$) and CD4+ ($p < 0.01$) T cells as compared to HD. The expression of NKp46 and CD107a positively correlated ($p < 0.05$). The expression of nectin-2 and MIC-A on *in vitro* activated CD4+ T-cells compared to receptor expression on NK cells (DNAM and NKG2D, respectively), was higher in non-AIDS patients compared to AIDS ($p < 0.05$).

Conclusion: The innate immune response of AIDS and non-AIDS patients differs despite similar CD4+ counts. NK cell activation and differences persist throughout treatment. Increased receptor-ligand expression in non-AIDS patients suggests an involvement of innate mechanisms, rather than CD4+ absolute counts alone, in preventing the occurrence of AIDS-defining opportunistic infections.

PE26

DC infected by the ANRS MVA_{HIV} vaccine candidate primes NK cells with anti-HIV specific activity through a mechanism involving NKG2D and NKp46 on NK cells, and membrane-bound IL-15 on DC

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Background: Natural Killer (NK) cells are the major antiviral effector cell population of the innate immune system. It has been demonstrated that NK cell activity can be modulated by the interaction with dendritic cells (DC). The vaccine candidate Modified Vaccinia Ankara encoding an HIV polypeptide (MVA$_{HIV}$), developed by the French National Agency for Research on AIDS (ANRS), has the ability to infect DC and to prime T cells. However, whether or not MVA$_{HIV}$-infected DC are able to induce anti-HIV specific NK cell activity remains undetermined.

Methods: DC were infected by MVA$_{HIV}$, or MVA$_{WT}$ vector as control, and co-cultured with autologous NK cells for 4 days. Then, NK cells were transferred to a plate containing recently infected DC or CD4+ T cells, and p24 production was determined at days 7 and 10 post-infection by ELISA test and flow cytometry. The implication of NK cell receptors NKG2D and NKp46, and membrane-bound IL-15 (mbIL-15) on MVA-infected DC, during the priming of NK cells was determined by using blocking mAbs.

Results: We found that NK cells primed by MVA$_{HIV}$-infected DC are significantly better at controlling HIV-1 replication in autologous DC and CD4+ T cells as compared to those primed by MVA$_{WT}$-infected DC. The specificity of anti-HIV NK cell activity was determined by measuring the NK cell activity against CMV-infected DC and target cells. In depth analysis of the priming showed that blockade of NKG2D and NKp46 during the priming induced decreased and increased anti-HIV-1 NK cell activity, respectively. Blockade of NKG2D during priming of NK cells by MVA$_{HIV}$-infected DC endowed NK cells with a particular NK cell receptor repertoire, with a marked decreased expression of activating receptors, and resulted in lower expression of mbIL-15 on MVA-infected DC; whereas blockade of NKp46 resulted in increased expression of mbIL-15 on MVA-infected DC.

Conclusions: These data demonstrate that the MVA$_{HIV}$ vaccine candidate is able to induce a specific anti-HIV-1 NK cell activity following their interaction with MVA$_{HIV}$-infected DC, and that the acquisition of such antiviral activity relies on a modulated crosstalk involving NKG2D and NKp46 on NK cells and the regulation of mbIL-15 on infected DC.

A22 – Monocytes and macrophages

PE27

HIV-1 stimulates Human Monocytes to produce BAFF through Type I IFN and its secretion level shows sex-related differences

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Background: B-cell-activating factor (BAFF) is a TNF ligand superfamily protein mainly expressed by myeloid cells. It is a potent regulator of B-cell development and function; however its overexpression has been associated to several B-cell dysfunctions. BAFF has been found to be increased in HIV-1-infected patients, suggesting its possible role in the B-cell dysfunction observed during HIV-1 infection. The exact mechanism by which HIV-1 infection leads to increased BAFF secretion is still unknown. We hypothesized that HIV-1 could indirectly influence the functionality of B-cells by increasing BAFF production on monocytes, one of the main sources of this cytokine.

Methods: PBMC were isolated from blood samples of healthy, informed and consented volunteers (n=9; 5 men and 4 woman). Monocytes were purified from PBMC by magnetic separation and stimulated with fully infectious NL4-3(X4) or NL4-3Balenv(R5) virus, Poly I:C or IFN-α2a, or mock control for 24h. In some experiments, monocytes were pre-incubated with B18R receptor before stimulations to neutralize type I IFNs. BAFF protein secretion was evaluated using a commercial ELISA kit. Type I IFN activity was monitored using HEK293-blue IFN-α/β sensor cells in monocytes supernatants. Statistical differences were determined by one-way ANOVA and two-tailed student's t-test.

Results: BAFF secretion was significantly increased in supernatants from monocytes stimulated with NL4-3 and NL4-3Balenv compared to mock, similar results were obtained with Poly I:C and IFN-α2a after 24h (n=9; $p < 0.05$). Both viruses induced a strong type I IFN response (n=9; $p < 0.05$). Neutralization of secreted type I IFN with B18R receptor decreased BAFF secretion to basal levels in monocytes stimulated with X4 and R5 HIV-1, Poly I:C and IFN-α2a (n=6; $p < 0.05$). BAFF secretion was higher in monocytes obtained from women (n=4) than those obtained from men (n=5) ($p < 0.05$).

Conclusion: Our results demonstrate that fully infectious HIV-1 can directly increase BAFF secretion on human monocytes via type I IFN and its secretion levels are sex-dependant. This study will help to elucidate the mechanisms of BAFF augmentation and B-cell disorders observed in HIV patients. This information will be relevant for the future design of therapies that could restore the normal functionality of the B-cell compartment.

HIV-1 virion accumulation and ATP-Induced release in human primary monocyte-derived macrophages

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Background: Macrophages are targets of HIV-1 infection and represent viral reservoirs in individuals receiving cART. A peculiarity of macrophage infection is their capacity to store new progeny virions in intracellular vacuolar compartments of debated origin. Moreover, functional polarization of macrophages towards the pro-inflammatory M1 or anti-inflammatory M2 cells restricts HIV-1 replication by different mechanisms (E. Cassol et al., J. Immunol. 2009). Therefore, we investigated whether human primary monocyte-derived macrophage (MDM) polarization involved the accumulation and release of HIV-1 virions upon acute infection and stimulation with extracellular ATP.

Methods: Human MDM were obtained from purified monocytes of HIV seronegative donors and were polarized (or not) into M1- or M2a-MDM as published (ibidem) and infected either with an R5 HIV-1 strain or with a VSVg-pseudotyped vector expressing eGFP. The cells were then stimulated or not with ATP (known to induce IL-1b release from intracellular compartments) in order to induce the potential release of virions from intracellular vesicles. In addition, the U937-derived promonocytic cell line U1, chronically infected with HIV-1, was differentiated to macrophage-like cells by PMA and was then stimulated with urokinase-type plasminogen activator (uPA) to favor retention of virions (M. Alfano et al. Blood, 2009) and then exposed to ATP to induce their release. Virion production was evaluated by the RT activity assay; other techniques were applied when appropriate.

Results: Extracellular ATP induced HIV virion release from both unpolarized and polarized HIV-infected MDM and in PMA-differentiated U1 cells stimulated with uPA. Virion release in MDM was associated with a reduction of intracellular virion retention, as measured by intracellular p24 staining and FACS analysis. We are currently analyzing virion morphogenesis and subcellular localization in polarized vs. unpolarized MDM and we are exploring additional pathway stimulating or preventing virion release in these cells.

Conclusion: ATP stimulation led to induction of preformed virion release in both primary MDM and in PMA-differentiated U1 cells stimulated with uPA. The identification of potentially "druggable" targets leading to either retention or release of preformed virion release by macrophages could be of significant relevance for identifying novel strategies of purging HIV-1 reservoirs in individuals under cART.

A24 – Antibody diversity and function

PE29

Generation and characterization of neutralizing anti-V3 scFvs against HIV-1 clade C

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Background: Majority of the HIV-1 viruses infecting Indian patients and more than 50% of the infections worldwide belong to clade C. An effective vaccine against HIV-1 should elicit bNAb responses against a series of diverse HIV-1 strains. The third variable region (V3) of HIV-1 is highly conserved and allows HIV-1 binding to host cells via the HIV-1 coreceptors. In this study we have successfully generated two neutralizing anti-V3 scFvs.

Methods: An antigen specific scFv phage library was constructed from the enriched V3-positive antibody secreting EBV transformed cells of a drug naive HIV-1 clade C infected Indian patient whose plasma exhibited high neutralizing potential against a panel of viruses and also displayed cross-reactive anti-V3 antibodies. Two anti-V3 scFvs were selected after biopanning and checked for their antigen binding specificity in ELISA. The scFvs were checked for soluble expression and purified using metal chelate chromatography. The purified scFvs were checked for their neutralization potential in TZM-bl based assay. Preferential antibody gene usage of these scFv were determined by DNA sequencing

Results: We generated two anti-V3 scFvs, 1E7B and F2C from an antigen specific phage library. The anti-V3 scFvs were expressed (32 kD) and confirmed by SDS-PAGE and Western blot. The soluble scFvs were highly specific to their antigens and did not show any reactivity against other unrelated peptides. The scFvs showed varying degrees of neutralization against 5/5 tier 1 and 7/12 tier 2 viruses. The two scFvs showed cross neutralizing activity against clade A, B and C viruses. The gene usage of scFv 1E7B and F2C was determined to be IGHV4-31*03 and IGHV4-31*02 genes in heavy chain and IGKV3-20*01 and IGKV2-28*01 in light chain respectively.

Conclusion: Our study suggests that the anti-V3 scFv generated from clade C infected Indian patient display varying degrees of neutralization potential against tier 1 and 2 viruses. Further defining the epitope specificities of these anti-V3 scFvs will be helpful in identification of epitopes, unique to clade C or shared with non-clade C viruses, for immunogen design and will serve as a prerequisite for designing a polyvalent vaccine against a broad spectrum of HIV-1.

PE30

Antibody and T cell response to the protease cleavage sites drive extensive mutations and correlated with protection against higher dose of SIVmac239 challenge and disease progression in Cynomolgus macaques

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Background: The protease of HIV-1 is a small 99-amino acid aspartic enzyme that mediates the cleavage of Gag, Gag-Pol and Nef precursor polyproteins. The process is highly specific, temporally regulated and essential for the production of infectious viral particles. A total of twelve proteolytic reactions are required to generate a viable virion. Since the protease cleavage sites of HIV-1 are highly conserved among major subtypes of HIV-1, direct immune responses against these cleavage sites would destroy the virus before its permanent establishment in the host. The vaccine could also force the virus to accumulate mutations around these sites and eliminate the normal function of the HIV protease thus eliminating infectous virions. Therefore a HIV vaccine targeting protease cleavage sites could be effective.

Methods: We have conducted a proof of concept study to investigate the feasibility and effectiveness of this vaccine approach. The recombinant VSV-peptides were used to immunize cynomolgus macaques and nanopackaged peptides were used to boost the immune response to the peptides overlapping the 12 protease cleavage sites of SIVmac239. The immunized macaques and controls were cumulatively challenged intrarectally with increased dosage of SIVmac239. Antibody and T cell responses to the peptides, SIVmac239 infection and plasma viral load, CD4+ and CD8+ T cell counts were monitored.

Results: Antibody and T cell responses to the 12 protease cleavage sites can protect macaques against higher dosage of SIVmac239 intrarectal challenge ($p=0.005$, R=0.42) and the vaccine group maintains significantly higher CD4+ counts ($p=0.0002$) than the controls weeks after being infected. Viral sequence analysis detected extensive mutations in the PCS and the flanking region. The break-through viruses of the vaccine group have higher mutation rate in PCS regions than that of the control group. The extensive mutations around PCS sites correlated with lower viral load ($P< 0.0001$).

Conclusion: A HIV vaccine targeting sequences around the 12 protease cleavage sites can be used to prevent HIV-1 infection and to treat HIV-1 infected patients.

A27 – Cellular immunity

PE31

Assessing the Role of Antigen-Specific CD8+ T cells in Delayed Progression to AIDS

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Background: One major challenge in the creation of an HIV-1 vaccine is the extreme genetic diversity of the virus. It is thought that a more cross-reactive T-cell response would be beneficial in circumventing this issue. Despite this, little is known about the characteristics of these responses in nature, and many aspects of the variant-epitope CD8+ T-cell response remain poorly defined. Here, we characterize CD8+ T-cells specific to 4 immunodominant HIV-1 epitopes, and their common variants, to better understand the level of cross-reactivity between them, and how this changes over time with progression to AIDS.

Methods: PBMC samples were collected from HIV+ female commercial sex workers from Nairobi, Kenya. Samples were stimulated for 6 hours with HIV-1 Gag and Envelope peptides, and IL2, IFNy, TNF, and MIP1B were assessed via intracellular flow cytometry. Each sample was also stained with tetramers specific to each stimulating peptide to assess which cells were actively secreting the cytokines. Samples were collected from multiple time points in the same patients over 1-6 years when available.

Results: Intracellular cytokine and tetramer staining revealed that the vast majority of cytokine production was by CD8+ T cells specific to the stimulating peptide. In some cases, cross-reactivity existed between epitopes and their variants, indicating that those regions may be better targets for future therapeutic agents. IL2 production was found to be absent or very low in nearly all patients; however, it was found that IL2 production in response to PMA was primarily in a subset of $CD8^{lo}$ cells, unlike the other cytokines, which were more likely produced by the $CD8^{hi}$ population. Cytokine production varied greatly in individual patients over time during progression to AIDS.

Conclusion: Considering the ease with which HIV-1 mutates, it is important to consider how effective CD8+ T cell responses are to these common HIV variants, and how they may change as disease progresses. This study provides unique insight into not only how responses to these variants differ, but how they change throughout long-term HIV infection. A better understanding of the dynamics of these important responses will be essential in guiding future vaccine or therapeutic candidates.

PE32

HIV-1 Env-specific conformational ADCC epitopes: potential utility for HIV vaccine design

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Background: HIV vaccines based on neutralizing antibodies and cytotoxic T-cells have been unsuccessful. The partially successful RV144 HIV vaccine trial demonstrated an important role of binding antibodies that mediate Antibody Dependent Cellular Cytotoxicity (ADCC). We previously identified and mapped HIV-1 linear ADCC epitopes in HIV+ subjects using HIV-1 Env overlapping peptides. However, ADCC antibodies to conformational Env epitopes are likely to recognize more conserved regions and therefore most useful in the design of novel HIV vaccines.

Methods and Results: We initially screened plasma samples from 22 HIV+ subjects with progressive HIV infection for ADCC responses against HIV-1 Env overlapping peptides and trimeric gp140 protein by using NK cell activation ADCC assay (IFNγ+ CD107a+) to identify the potent ADCC epitopes. We observed that 6 out of 22 (27%) HIV+ subjects had ADCC responses to trimeric gp140 (mean NK cell activation 2.8%, range 1.2-5.1) that were absent when tested against HIV-1 Env overlapping peptides, indicating ADCC response to probable conformational Env epitopes. When we tested plasma samples from 3 long-term slow progressors (LTSP, CD4 T cells>500/µl for >10 years), 2 out of 3 subjects (66%) had ADCC response to trimeric gp140 (mean NK cell activation 5.1%, range 4.8-5.4) and much stronger responses to monomeric gp120 (mean NK cell activation 16.1%, range 15.8-16.4) were observed, suggesting relevant immunologic differences between monomeric and trimeric Env. We also tested plasma samples from 4 elite controllers (EC, viral load consistently < 400 off ART), 2 out of 4 (50%) had ADCC responses to trimeric gp140 (mean NK cell activation 1.1%, range 0.6-1.6) and 3 out of 4 (75%) had ADCC responses to monomeric gp120 (mean NK cell activation 3.9% range 2.0-3.5).

Conclusion: Our results suggest that HIV+ subjects who naturally control HIV have robust ADCC responses to conformational Env epitopes. Dissecting the specific conformational ADCC epitopes by using a panel of native, truncated, modified and chimeric Env proteins will help in designing new HIV antibody vaccine immunogens. We are now recruiting additional subjects and studying ADCC responses to different HIV-1 subtypes to identify common ADCC epitopes that would help in designing globally relevant immunogen.

PE33

HIV-1 infection reduces emigration of mature thymocytes from the human thymus by downregulation of the sphingosine-1-kinase receptor S1PR1

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Background: Sphingosine-1-phosphate (S1P) is a chemotactic sphingolipid molecule present at high levels in blood, but low levels in lymphoid and other tissues. In the mouse it has been shown that S1P plays an important role in the egress of lymphocytes from the lymphoid tissues to peripheral blood. We investigated the dynamics of S1P and its five known receptors, S1PR 1-5, in the context of egress of naive T cells from the human thymus to the periphery, which is thus far not well understood. We hypothesized that thymocytes migrate toward S1P when ligated to S1PR1, and that due to interactions between CD69, Interferon alpha and HIV-1, that HIV infection would downregulate S1PR1.

Methods: To examine the kinetics of migration in response to S1P, we performed migration assays in transwell-membrane plates with various concentrations of S1P in the presence or absence of agonists and antagonists to S1P. To determine which S1P receptors are expressed on developing T cell subsets in the thymus we performed real-time PCR and 9-color flow cytometry on fetal, postnatal and thymic implants from NSG thy/ liv mice to verify the expression of S1PR1-5 on thymocytes. We additionally performed *in vitro* and *in vivo* HIV-1 infection of thymocytes with X4- (NL4-3) and R5- (JR-CSF) tropic virus.

Results: Our results show that S1PR1 is significantly upregulated in mature thymocyte subsets about to exit the thymus to the periphery as naïve T cells. Thymocytes migrate to S1P and FTY720 inhibits migration by functioning as a S1P analogue to downregulate of one or several of the S1P receptors: S1PR1, 3, 4, 5. *In vitro*, S1PR1 expression is decreased in mature naïve thymocytes 2d post infection. This effect is no longer observed at two weeks. *In vivo* infections are currently in progress to confirm this effect.

Conclusion: HIV-1 infection perturbs the natural egress of mature thymocytes from the thymus via downregulation of S1PR1, the primary S1P receptor for thymic egress. As T cell reconstitution is a constant challenge in HIV infection, this discovery could have significant impact on how we approach therapeutics targeting T cell regeneration.

A28 – Mucosal immunity

Severe depletion and exhaustion of mucosal-associated invariant T (MAIT) cells in HIV-1 infected patients

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Background: Mucosal-associated invariant T (MAIT) cells compromise an evolutionarily conserved class of T-cells that act as a bridge between innate and adaptive immunity. They are characterized by the expression of the semi-invariant TCR Vα7.2 that recognizes antigens presented by MHC class I related (MR1) protein. Their ability to be stimulated by bacterial-metabolism derived B-vitamin-derivatives suggests a role in host defence against bacterial pathogens.

Methods: We studied the frequency and distribution of MAIT cells defined as CD3+CD4-TCRγδ-CD161++ lymphocytes in different groups of HIV patients and healthy controls.

Results: We show that MAIT cells are drastically depleted from the peripheral blood of HIV-infected patients. Their frequency is reduced from 6.38% (mean frequency of CD3+CD4-TCRγδ- lymphocytes) in healthy individuals to 0.87 % in HIV infected patients with a viral load of >100.000 copies/ml (p< 0.0005). They are not reconstituted in patients receiving antiretroviral treatment (0.98%) and only slightly less depleted in elite controllers (1.41%). Moreover, residual MAIT cells in HIV-progressors show a significantly elevated expression level of the exhaustion marker PD-1 (34.62% of MAIT cells are PD-1+ in HIV patients with VL>100.000 compared to 23.17% in healthy donors, p=0.018). MAIT cells from elite controllers show a nonexhausted phenotype (25.13%) and the exhaustion is partially reversed in HAART treated patients (28.61%).

Conclusion: The loss and exhaustion of MAIT cells could have important implications for the mucosal defence against bacterial pathogens, particularly in the gut of HIV-infected patients.

A29 – Viral determinants of pathogenesis

PE35

NEF-mediated down-regulation of MHC I expression in thymocytes may affect the pathogenicity of a pediatric isolate of X4 HIV-1

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Background: An X4-tropic primary pediatric HIV-1 isolate PI-2 was found to be less pathogenic for developing thymocytes than the related primary isolate PI-2.1 and the X4-tropic laboratory isolates NL-4-3 and NF-NSX. HIV regulatory genes were analyzed for mutations and function to determine the reason for a relatively low pathogenicity. Previously, Nef-mediated CD4 downregulation, but not MHC-I downregulation, has been linked to pathogenicity. We examined the Nef gene of PI-2 to assess its role in pathogenicity.

Methods: We compared the Nef gene of PI-2 with that of more pathogenic strains (NL4-3 and PI-2.1) and assessed MHC-I and CD4 expression in infected thymocytes by flow cytometry previously transfected. Pathogenicity was assessed using in vivo infection of thy/liv implants in SCID-hu mice. PI-2 nef gene was placed into a HIV-1 genome construct lacking env and vpu to establish that differential modulation of MHC expression by PI-2.1 was nef-mediated.

Results: Novel mutations affected 16 amino acids in nef, including a 7 amino acid deletion (ΔK7S) in PI-2. KC57+ thymocytes productively infected with PI-2 expressed higher levels of MHC-Class I (HLA-ABC) than the same cells infected with PI-2.1 or NL4-3, but levels of CD4 expression were similar in all cases. PI-2 infected thymocytes were less markedly depleted of CD4+ cells than thymocytes infected with PI-2.1 or NL4-3, suggesting that MHC-Class I downregulation may be related to cytopathicity. MHC-Class I downregulation was also not observed in productively infected (KC57+) thymocytes after infection with less cytopathic pediatric isolate PI-2 in vivo thy/liv implants in SCID-hu mice. Nef, but not Env or Vpu-mediated MHC-Class I downregulation, was impaired when a VSV-pseudotyped virus contained the mutated *nef* gene from the less cytopathic isolate PI-2.

Conclusions: The less pathogenic pediatric isolate (PI-2) contains novel mutations in *nef.* Our *in vitro* and *in vivo* data with primary isolates suggest that MHC-I downregulation plays a larger role in pathogenicity than observed using highly cytopathic laboratory strains such as NL4-3 The impact of mutations should therefore be investigated in primary isolates rather than in molecularly cloned isolates.

A30 – Acute and early HIV/SIV infection

PE36

Analysis of HLA-restricted HIV-specific CD8 T cell responses and viral adaptation in patients with acute HIV infection prior to full seroconversion

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Background: Identification of the earliest post transmission HIV-specific CD8 T cell responses and viral adaptation to them is important for both vaccine design and early treatment or eradication strategies. Here we describe the first CD8 T cell responses demonstrated in 9 samples collected from 7 individuals experiencing acute HIV infection.

Methods: HLA-restricted HIV-specific CD8 T cell responses were evaluated by IFNγ-ELISpot assay. HIV full genome sequences were determined in 3/7 patients using 454 deep sequencing. Samples were collected within a median of 1 month (range 7 days to 2 months, n=7) post HIV-seroconversion illness and a median of 19 days (range 3-52 days, n=5) post detection of any HIV antibody. All patients were studied before full seroconversion on Western Blot, in Fiebig stage IV.

Results: 36 HIV-specific CD8 T cell responses were detected (median 4 [range 1-13] responses) from a median of 56 (range 33-71) peptides evaluated per patient. HIV Gag stimulated the highest frequency IFNγ responses (n=17), followed by Nef (n=7), Pol (n=5), Vpr (n=4), Vif (n=2) and Env (n=1). Gag peptides also elicited the highest single magnitude IFNγ peptide response in 5/7 patients (median 800 [range 250-3210 SFU] n=5). Responses broadened from 1 to 6 in one patient tested 3 and 14 days post WB but were similar in a second case tested 25 and 39 days post WB. Amino acid changes (compared with HXB2) were observed in 7/10 recognised T cell epitopes in three patients with HIV sequence available. One amino acid change was located in a known site of viral adaptation in HIV Nef. An additional amino acid change was observed in a non-recognised T cell Nef epitope in a known viral adaptation site.

Conclusion: HIV-specific IFNγ responses were detected in 7 cases of acute HIV infection prior to full seroconversion. Responses were narrow and variable in magnitude with the majority of responses targeting HIV Gag. Early viral adaptation pre complete seroconversion was demonstrated. These data have implications for preventing viral adaptation using early antiretroviral treatment or eradication strategies, as well as for design of preventative vaccines.

A41 – Elite controllers

PE37

An elite controller in pregnancy - towards a definition of recovery, though not cure?

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Background: An elite controller has been defined as "having persistently undetectable plasma RNA for 15-20 years, with stable CD4 counts, without therapy". Low/undetectable levels of proviral HIV DNA are also a feature. We describe a case of a 22-year-old woman with evidence of anti-HIV antibodies but no detectable virus.

Methods: Blood was collected from mother and baby for diagnostic assays.

Results: An asymptomatic, 22-year-old pregnant Indian woman presenting for routine antenatal screen at 12-weeks gestation was found positive for HIV antibodies (Abbott Architect HIV Ag/Ab Combo). Her CD4 count was 551 cells/ microlitre and HIV RNA was undetectable (< 40 copies/ml, Abbott Real-Time HIV-1 RNA). Her medical history included mild-intermittent asthma and genital warts. Confirmatory testing showed the following positive bands on Western blot (Genelabs, MP Diagnostics HIVBlot 2.2): p24, gp41, gp120, gp160, confirming a positive HIV status. Further supplementary testing demonstrated the presence of anti-HIV antibodies only and no p24 Ag (VIDAS DUO ULTRA HIV5/P24II, Biomerieux) and a proviral HIV DNA assay was negative on whole blood (Roche Amplicor HIV-1 DNA Test v 1.5). Since these results were surprising, we checked her CCR5delta32 status using an in-house assay, which showed that she was wild-type for this allele. Despite the absence of detectable HIV-1 RNA, she received zidovudine, lamivudine and boosted-lopinavir from gestational week 16 until delivery. She delivered a full-term, healthy baby, by Cesarean section and abstained from breast-feeding as advised. There was no evidence of HIV infection in the infant until at least 12 months after delivery, as indicated by HIV-1 RNA and HIV-1 DNA viral load and anti-HIV/p24Ag serological testing. Throughout her pregnancy and beyond, the woman's white cell markers remained within, or exceeded the normal limits.

Conclusion: This case does not meet the definition of an elite controller quite yet because 15-20 years have not yet passed. Only one convincing case of HIV clearance has been documented - an HIV-1 infected patient who underwent stem-cell transplantation for acute myeloid leukemia from a CCR5delta 32 homozygous donor. Such cases raise interesting questions as to what we might consider as the criteria for defining HIV *recovery* versus *clearance*.

PE38

HIV-1 Vif- and Nef-responses in controllers present higher magnitude and target more conserved, less promiscuous epitopes than responses seen in typical progressors

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Background: Understanding on the protective cellular immune responses seen in HIV-1-infected controller individuals has been believed to be important in order to delineate successful vaccination strategies for HIV-1 control. Here, we explore cellular immune responses against Vif and Nef in 29 HIV-1-infected subjects presenting durable control of viremia and compared them to responses seen in eight typical progressors.

Methods: ELISPOT-IFN-γ assays were carried out using 15-mer peptides, overlapping by 11 amino acid, encompassing the whole sequences of HIV-1 consensus B Vif and Nef proteins. On the 15 amino acid sequences targeted by controllers or by progressors, we characterized *i*) the intra-subtype B genetic variation, *ii*) the number of potential HLA-I *loci* A and B-restricted epitopes, and *iii*) the number HLA-I *loci* A and B molecules that potentially bind to each epitope in the 15-mer sequences by assessing *in silico* data from the Los Alamos HIV database.

Results: Collectively, our data indicate that responses mounted by controllers have same breadth of targets, but are of higher magnitude than responses presented by progressor individuals. Furthermore, after investigating more specifically the responses showed exclusively by controllers or by progressors, we observed that controllers target viral sequences more conserved and less promiscuous to HLA-I binding. Vif- and Nef-sequences targeted by controllers seem to be less exposed to cellular immunity-mediated selective pressure, as the number of HLA-I molecules that could recognize an epitope on them is lower. This is reflected by correspondent lower genetic variation we observed in these sequences.

Conclusion: These findings suggest that different amino acid sequences, in the viral proteome, are able to elicit host cellular responses with differential impact on viral control. We believe that the identification of such phenomenon provides clarification about the nature of the successful cellular responses against HIV-1, seen in controller individuals.

A44 – Highly exposed seronegative individuals (HESN)

PE40

Polymorphisms in the IRF1 gene associated with reduced HIV susceptibility and their impact on plasma and cervical lavage cytokine/chemokine expression

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Background: Interferon Regulatory factor 1 (IRF1) is a transcriptional activator of interferon genes and interferon inducible genes and plays a crucial role in host antiviral immunity and HIV replication. Previous work has shown association of three polymorphisms in IRF1 with decreased acquisition of HIV-1 infection in a Kenyan female sex worker cohort. Peripheral blood mononuclear cells from individuals with protective IRF1 genotypes show reduced endogenous IRF1 expression and impaired transactivation of HIV-1 LTR when infected with a single-cycle HIV-1 VSV-G pseudovirus construct expressing a luciferase reporter gene insert, suggesting a limited ability to support HIV replication. This study will characterize the effect of the identified IRF1 polymorphisms on plasma and cervical lavage (CVL) cytokine/chemokine expression, focusing on IRF1's function in regulating the expression of host immunological genes and the impact this may have on susceptibility to HIV-1 infection.

Methods: We investigated the effect of IRF1 polymorphisms on the expression of 22 different cytokines/chemokines (IL1β, IL2, sIL2Rα, IL6, IL8, IL10, IL12p70, IL15, IL17, sCD40L, Fractalkine, IFNγ, IP10, MCP1, MCP3, MIP1α, MIP1β, TNFα, ITAC, MIG, MIP3α and MIP3β) in plasma and CVL samples from female sex-worker cohort from Nairobi Kenya. Cytokine/chemokine levels were measured using Miliplex MAP multiplex kit (Human Cytokine/chemokine panel I and III from Millipore, Billerica, MA) and analyzed on the BioPlex-200 (Bio-Rad, Mississauga, ON, Canada).

Results: We observed significantly increased expression of IL15, IL17 and IFNγ, and decreased expression of MIP1α in the plasma samples of individuals with protective IRF1 genotypes. Additionally, we observed significantly higher expression of IL2, IL15 and IFNγ in CVL samples from individuals with protective IRF1 genotypes, compared to the individuals without the protective IRF1 genotypes.

Conclusion: The observed increase in anti-viral cytokine expression in the plasma and CVL from the female sex workers with protective IRF1 genotypes could represent an important mechanism in preventing the establishment of HIV infection. It is important to fully characterize the effect of IRF1 polymorphisms as this will further the understanding of natural resistance to HIV infection and can contribute to the development of novel prophylactic or therapeutic modalities.

A45 – Correlates of protection

PE41

Functional cure after long term HAART initiated during early HIV infection - a comprehensive case study

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Background: Early initiation of cART during acute HIV infection can lead to control of viral replication after cessation of therapy in a rare subgroup of patients termed post treatment controllers (PTC). We set out to define immunological and virological correlates of post treatment control and to assess the potential of eradication vs. functional cure.

Methods: A 67 yrs. old male was treated with cART 3 months after HIV exposure and 1 month after seroconversion for a total of 5,5 yrs.; cART was stopped in May 2004 and the patient remained BLOD (< 20 c/ml) and shows normal T cell counts and distribution without ART since 9 years. We performed comprehensive analyses to assess the immuno-virological correlates of PTC including a humanized mouse model in this patient.

Results: CD4 count is stable between 900-1000 cells/µl, the homozygous CCR5 promoter variant A59029G but no delta 32 deletion was detected, HLA-I subtype was A 01, 02 B: 44, 52; no viral RNA or DNA was detected using ultrasensitive techniques (single copy assay, viral co-culture, DNA-PCR) in plasma, PBMC and CSF. No p24 antigen or HIV-RNA was detected in gut biopsies by immunohistochemistry or in situ hybridisation. ELISPOT revealed strong polyfunctional CTL responses against gag and nef epitopes and polyfunctional HIV specific CD4 responses and a normal distribution of TEM and TCM comparable to a control group of nine elite controllers (EC) was shown. The frequency of peripheral Treg and Th17 cells was comparable to normal controls and EC. Virus could be recovered in vivo in a humanized mouse model after transplantation of purified donor CD4 T cells and anti CD3/CD28 stimulation indicating the persistence of replication competent virus.

Conclusion: The data obtained in this unique case suggest a functional cure of this patient rather than viral eradication after early onset cART. The presence of strong HIV specific T cell responses, normal frequency of regulatory T cells and animal data suggest a strong role of preserved adaptive immune responses as a correlate of viral control in this patient arguing for adjuvant immunotherapeutic interventions (e.g. therapeutic vaccination, Il-7) in this setting.

A46 – HIV drug development

PE42

Evaluation of a panel of selective inhibitors of PKCθ or Lck for controlling HIV-1 replication in CD4+ T cells

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Background: PKC theta (θ) is selectively expressed on CD4+ T lymphocytes and is activated through TcR/CD28 engagement at the immunological synapse. PKCθ activation is mediated by the lymphocyte-specific protein tyrosine kinase (Lck), which is required for PKCθ translocation to the plasma membrane. This initiates a cascade of events that culminates in the activation of essential factors for HIV-1 replication such as ERK, AP-1 and NF-κB. We reported previously that blocking PKCθ selectively by rottlerin reduces viral replication in T lymphocytes and that mRNA interference for PKCθ provided a refractory state to HIV-1 infection. Now we analyzed the effect on viral replication of a panel of 10 selective PKCθ or Lck inhibitors and proved that blocking Lck also reduced HIV-1 replication.

Methods: 10 selective PKCθ or Lck inhibitors were used to evaluate their ability to control HIV-1 infection in PBLs and MT-2. Inhibition of viral replication was determined by infecting with NL4.3-renilla (X4) and BX08-renilla (R5) strains. IC_{50} and CC_{50} were estimated using GraphPad Prism software (sigmoidal dose-response formula). Inhibition of NF-κB was monitored by transient transfection of κB-LUC expression vector. Proviral integration was analyzed by quantitative Alu-LTR PCR. Released IL-2 was assessed by ELISA.

Results: all PKCθ or Lck specific inhibitors reduced HIV-1 (X4- and R5-tropic) replication in PBLs and MT-2. Specifically, CGX0471 and CGX1079 (CompleGen) inhibited HIV-1 replication (NL4.3wt) more than 14-fold in MT-2 (IC_{50}=3.67µM and 2.28µM, respectively) and more than 16-fold in PBLs (IC_{50}=11.18µM and 12.86µM, respectively) (R^2=0.98; CC_{50}>50µM). Both compounds reduced NF-kB activity and the decrease of IL-2 release to the culture medium was assessed. Lck inhibitor II (Merck) also reduced HIV-1 replication in PBLs (IC_{50}=1.19µM; CC_{50}>37µM) and MT-2 (IC_{50}=15µM; CC_{50}>100µM), as well as IL-2 release, NF-κB activity and proviral integration.

Conclusions: PKCθ is essential for HIV-1 replication in CD4+ T-lymphocytes. Specific inhibition of PKCθ and Lck in CD4+ T-cells delayed HIV-1 transcription and proviral integration. Since both kinases are expressed primarily in T-lymphocytes, the use of specific inhibitors as adjuvant of cART during acute infection may reduce the pool of activated CD4^{+} T-cells, the viral production and the size of the reservoir, without causing general immunosuppression.

PE43

Safety and Efficacy study of a herbal concoction used for alternative/complementary treatment of HIV infection in Nigeria

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Background: It is not uncommon for patients to opt for orthodox remedies exclusively or concurrently with modern remedies for disease conditions like HIV-AIDS. This study was designed to evaluate the safety in experimental animals and efficacy in human of an herbal concoction, 'α-Zam' used by clients seeking herbal remedy for treatment of HIV infection in Nigeria.

Methods: Graded doses (400mg/kg, 800mg/kg, 1600mg/kg and 3200mg/kg) of α-Zam were administered to 4 test groups of Wistar albino rats for 84 days; the control received distilled water. On the 85th day paired blood samples were collected from all the rats for haematology and clinical chemical parameters. Preliminary medical and laboratory examinations using WHO criteria were done after confirmation of HIV infection by Western blotting in the nearest teaching hospitals to the residence of fifty-one patients (23 males, 28 females) taking α-Zam as complementary or alternative therapy. The patients were studied for 16 months to assess the adverse-effects, drug interactions, toxicity and effectiveness of the herbal remedy using blood microscopy, sputum AAFB, urine microscopy, haematology, clinical chemistry and viral (HIV-RNA) load by means of Polymerase Chain Reaction (PCR).

Results: In rats, this herbal remedy caused significant increase in globulins at 1600 mg/kg and 3200 mg/kg (61g/dl and 62 g/dl) compared with the control group (56 g/dl). About 7.8% of the patients had average increase in CD4 count of 262±16 cell/μL, 45.1% patients with average increase of 310±16 cell/μL, 31.4% patients with average increase of 456±25 cell/μL and 15.7% patients with average increase of 510±36 cell/μL post-treatment. There was very marked reduction in viral (HIV-RNA) load with 80.4% (41.3 % females, 39.1% males) and19.6% (12.1 % females, 7.5 % males) HIV infected patients having undetectable viral load and < 1000 copies/ml respectively after the therapy. There was no evidence of adverse drug interaction in the patients using both the herbal and highly active anti-retroviral therapy (HAART).

Conclusion: The herbal concoction is an effective anti-HIV agent by causing increase in CD4 counts and decrease in viral load with no adverse drug interaction with HAART and could be studied further for periodic immunologic and virologic post-therapy.

A47 – Mechanisms of anti-retroviral drug resistance

PE44

THE REAL CAUSE OF INCREASING HIV OPPORTUNISTIC INFECTIONS IN CHUK (KIGALI UNIVERSITY TEACHING HOSPITAL)

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Background: In spite of good and free of charge ARVs distribution among people living with HIV in Rwanda, nowadays, opportunistic infections among those people are more and more increasing which is suggestive of non controlled HIV infections. For that purpose a study intending to highlight what might be the main causes has been conducted.

Methods: We took randomly 75 female patients admitted in CHUK (main and vast hospital of Rwanda) diagnosed with HIV opportunistic infections despite of taking ARVs. The inclusion criteria were, all patients (no age limit) attending CHUK who started ARVs from 2008.

Results: : 47 of them were recognized with previous bad compliance to medications, 28 patients reported good adherence and shifted to second regimen of ARV due to clinical and immune failures:unfortunately,16 of 28 patients were seen with inappropriate selection of the second regimen ARVs).

Conclusion: It came up with bad compliance of patients to ARVs, possible mutated HIV and inappropriate choice of second regimen that induced to resistance, from that we recommend a strict follow up on ARVs adherence, and well trained ARVs providers.

PE45

Secondary resistance mutations in the R263K integrase inhibitor resistance pathway

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Background: HIV-1 resistance has been observed for all antiretrovirals tested so far, raising concerns about the long-term efficacy of these drugs. However, no resistance mutation against the strand-transfer inhibitor dolutegravir has been observed in treatment-naïve patients. *In vitro* selection studies performed in our laboratory demonstrated that, in the presence of dolutegravir the R263K mutation commonly emerges in integrase and is often associated with the secondary mutation H51Y. We have shown that R263K confers resistance to dolutegravir while the addition of H51Y to R263K further decreases HIV susceptibility to this drug. However, resistance correlated with a pronounced decrease in integration and viral replication. Although less common than H51Y, other secondary mutations such as M50I and E138K were selected in the presence of the R263K primary mutation. We further characterize the R263K resistance pathway by studying the effect of the M50I and E138K secondary mutations on HIV resistance to dolutegravir, as well as on the catalytic activity of purified recombinant integrase and on viral fitness.

Methods: The various relevant integrases were cloned into a vector for bacterial expression and mutated by site-directed mutagenesis. Recombinant integrases were purified and tested for strand-transfer activity in cell free assays, and Michaelis-Menten constants and maximal activities were calculated. The same mutations have been introduced into pNL43 HIV clones by site directed-mutagenesis and the resulting viruses were tested for resistance to dolutegravir and viral replication capacity in cell culture.

Results: Similar to the H51Y mutation, M50I and E138K fail to restore the catalytic activity of the R263K integrase, as well as the defect in viral replication associated with this latter mutation. However, the M50I and E138K secondary mutations have different effects on R263K than H51Y, with M50I influencing integrase interaction with viral DNA and E138K impacting the conformation of the catalytic site. Additionally, M50I confers a higher resistance level to dolutegravir than E138K.
Conclusion: None of the secondary mutations associated with R263K restores HIV integrase activity, integration, or viral replication capacity, suggesting that the R263K resistance pathway may be beneficial to patients receiving dolutegravir-containing regimens.

A49 – Nucleic acid based HIV and SIV therapy development

PE46

Characterization of a new use for acyclovir and tenofovir using human cervico-vaginal tissue *ex vivo*

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Background: HSV-2, one of the most common HIV-1 copathogens, establishes a synergistic interaction with HIV-1. Several clinical trials have shown that the anti herpetic drug acyclovir decreases HIV-1 viral load and delays HIV-1 disease progression. Similarly, CAPRISA004 reported that a vaginal gel containing 1% of the anti-HIV-1 drug tenofovir unexpectedly decreases the risk of HSV-2 acquisition by 51%. Using a system of cervico-vaginal tissue culture that reflects many of the *in vivo* tissue features, we evaluated the dual anti-HIV-1/HSV-2 activity of acyclovir and tenofovir.

Methods: Human tissues were infected *ex vivo* with HIV-1 (laboratory strains, primary isolates or NRTI-resistant HIV-1 variants), HSV-2 (HSV-2$_G$, HSV-2$_{MS}$) or with a combination of HIV-1 and HSV-2 variants and treated with acyclovir or tenofovir. HIV-1 and HSV-2 replication were respectively monitored by measuring HIV-1 p24$_{gag}$ or HSV-2 DNA accumulated in culture media.

Results: Acyclovir suppressed the replication of the laboratory X4$_{LAI.04}$ HIV-1 with an EC50 of 3 µM (95% Confidence Interval (CI):1.85-5.24). No statistical difference between the inhibition of primary isolates and AZT-resistant variant was observed (n=5). In contrast, the EC$_{50}$ for the 3TC-resistant virus (M184V) was four times higher than that of the parental HIV-1 isolate (n=5). Tenofovir suppressed HSV-2 replication with respective EC$_{50}$s of 14 µg/ml (CI:10-163) for HSV-2$_G$, and 19 µg/ml (CI:27-127) for HSV-2$_{MS}$. Tenofovir 66 µg/ml reduced HSV-2$_G$ and HSV-2$_{MS}$ replication by 87±12% and 91.7±3.2%, respectively ($p<$ 0.01). Accordingly with the *ex vivo* results, we found in a cell-free system that the triphosphate form of acyclovir was a direct inhibitor of the HIV-1 reverse transcriptase and that tenofovir-diphosphate was a direct inhibitor of the HSV DNA polymerase.

Conclusion: Using human tissue culture *ex vivo*, we showed that (i) acyclovir inhibits HIV-1 at concentrations that are clinically relevant and commonly achieved in plasma of patients treated with acyclovir or valacyclovir; (ii) tenofovir, at the concentration achieved in topical vaginal application (which is substantially higher than achieved upon systemic administration) acts as a double-targeted antiviral agent as well. These data explain the inhibition of HIV-1 in clinical trials using acyclovir and the inhibition of HSV-2 observed in the clinical trial using tenofovir (CAPRISA004).

A50 – Design of approaches targeting inflammation/immune activation

PE47

Persistent immune activation despite suppressive HAART is associated with higher risk for viral blips in HIV-1 infected individuals

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Background: Viral blips are thought to represent random biological variations around a steady state of residual HIV viremia and to lack clinical significance. However, blips may be the consequence of shedding from activated immune cells and persistent immune activation has recently been linked with increased morbidity and mortality. We aimed to assess the association of persistent immune activation and the occurrence of blips.

Methods: HIV infected patients from our outpatient cohort who developed a blip after having been on fully suppressive HAART for at least 180 days were matched with patients without blips according to duration of time of complete viral suppression (CVS), age, sex, and CDC stage. Domain averaged areas under the curve for CD3+, CD4+, CD8+, CD3+HLADR+, CD4+CD45RA+, CD16+CD56+CD3-, and CD19+ cells, as well as CRP levels were calculated from first date of CVS until index date and included in conditional logistic regression models. Adherence to HAART was assessed by measuring prescribed NNRTI or PI plasma levels in a sample of 57 patients.

Results: 82 Patients with a viral blip were matched with 82 controls. Mean age at blip was 47.2(SD 12.1) years, 80.5% of patients were male and 42.7% had CDC stage C disease in both groups. Viral blips occurred after a median of 14 months (IQR 8-34) of CVS. In the logistic regression, activated CD3+HLA-DR+ lymphocytes (OR 1.39 per 100 cells/µl,[95%CI1.12-1.72],p=0.003) and HAART initiation after 2007 (vs. before 2001, OR 0.23[0.08-0.69],p=0.005) were significantly associated with viral blips. In 7/23(30%) specimens from patients with blips and 13/34(38%%) controls, drug levels were below therapeutic concentration (p=0.55).

Conclusion: The occurrence of viral blips after suppressive HAART was associated with persistently elevated markers of T cell activation. Blips were not explained by decreased drug levels and may identify a subset of patients with higher immune activation and increased risk for HIV disease progression.

A51 – HIV-hepatitis virus interactions

Hepatitis B virus coinfections: Prevalence and CD4 profile among patients on comprehensive care services in Kenya

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Background: In resource-limited settings, HIV co-infections are not routinely tested for. Yet antiretroviral therapy (ART) is increasingly becoming available to a wider population requiring treatment. Since Human immunodeficiency Virus and Hepatitis B virus share common risk factors, co-infections are an important public health problem in areas of high prevalence for both the viruses. Testing for both infections is therefore important for expanded care and management especially among the HIV infected. In this study determination of seroprevalence of HIV/HBV co-infection and CD4 was carried out in Kenya.

Methods: A multi centre study involving 840 blood samples collected from patients attending comprehensive care clinics was carried out. HIV and HBV serostatus was determined using ELISA and Rapid tests while CD4 count was done using FACScan flow cytometer

Results: Among the 840 HIV infected individuals, 151(18%) patients tested positive for HBV. Co-infections were higher in females (62%) than males (38%) with the age categories between 22-45 years having the majority (76%) of the co-infected individuals. Most, (73%) of the patients had CD4 count below normal, with 40% having a count > 200 cell/m^3.Among the co-infected, 57 %(65) of them had counts below 200cells/m^3

Conclusion: In conclusion, there is need to include screening for hepatitis B in the repertoire of the tests done in comprehensive care clinics for HIV and AIDS.

A52 – Tuberculosis and other mycobacteria

PE49

Anti-mycobacterial activity is enhanced by blocking the Tim3-Galectin 9 interaction in HIV patients

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Background: T cell immunoglobulin and mucin-(Tim)-3 domain is an inhibitory molecule involved in immune tolerance, autoimmune responses, and antiviral immune evasion. CD4+ and CD8+ T cells express it at variable levels. High expression of Tim3 on T cells from HIV patients has been associated with an exhausted immune phenotype. However, it has also been demonstrated that the Tim3 interaction with its ligand Galectin-9 (Gal9) induces macrophage activation that results in killing of *Mycobacterium tuberculosis* (M.tb). We speculate that manipulation of the Tim3-Gal9 pathway can restore lymphocyte function to eliminate intracellular Mtb-infection.

Methods: We included 20 HIV patients and 20 healthy controls (HC). All samples were obtained in accordance with the Institutional Review Board's protocol. Tim3 and Gal9 expression were analyzed by flow cytometry. Monocyte-derived macrophages (MDM) were infected with Mtb (H37Rv; MOI=10:1). After infection, T cells or blocking antibodies were added. Four days after infection, cells were lysed and mycobacteria counted. Cytokines were analyzed by FlexSet.

Results: We identified that basal expression of Tim3 was elevated on CD8+ T cells from HIV patients (26±9.2%) *vs.* HC (14.8±6.9%) (p=0.001). No changes were observed in Gal expression. Suppression of intracellular bacterial growth was normalized to the amount of bacterial growth in the absence of T cells (= 100%). T cells from HIV patients led to a 59.9% reduction in bacterial growth vs. 70.6% on HC. Control of bacterial growth in HIV patients increased to 100% based on the antibody blocking studies. The opposite phenomenon was observed in HC. We identified that the antimicrobial effect was partially dependent on the production of pro-inflammatory cytokines critical to limit bacterial growth.

Conclusion: Our results established that modulation of the Tim3-Gal9 pathway upon treatment with specific blocking antibodies might recover cytokine production by macrophages and T cells accelerating antimicrobial immunity against M.tb.

B56 – Therapeutic vaccine and immune based therapy trials

cART reduces antibody-dependent cellular cytotoxicity to HIV: Implications for Therapeutic Vaccines

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Background: Combination anti-retroviral therapy (cART) has proven effective in the control of HIV infection but it cannot eliminate HIV and treatment is life-long. HIV-specific cytotoxic T lymphocyte responses decline following cART but alterations in other HIV-specific immune responses that may assist in clearing latent HIV infection, specifically antibody-dependent cellular cytotoxicity (ADCC), are unknown. We hypothesized that ADCC immunity may decline in HIV-infected subjects on cART, which has implications for therapeutic vaccines to control HIV infection.

Methods: A cohort of 49 cART-naive HIV-infected subjects in Thailand started cART at baseline. The median baseline CD4 count was 186 cells/µl (range: 10-350 cells/µl) and viral load was 4.9 log10 copies/ml (range: 2.9-6.3 log10 copies/ml). Longitudinal blood samples were collected up to 96 weeks (median CD4 count: 338 cells/µl range: 113-990 cells/µl; median viral load: < 40 copies/ml, range: 40-137 copies/ml). Serum samples were analyzed for ADCC-mediated killing against HIV-1 envelope protein subtype AE (Env/AE) targets using the RFADCC assay. ADCC antibody responses to Env/AE peptide pools were measured using the NK cell activation ADCC assay. The total HIV-specific antibody binding titers against Env/AE were measured by ELISA.

Results: A significant reduction in Env-specific ADCC-mediated killing (p< 0.0002) was observed between baseline (median: 11.8%, IQR: 7.7-14.1%) and week 96 (median: 7.7%, IQR: 3.9-10.3%) in the RFADCC assay. In the NK cell activation ADCC assay, an initial screening showed a reduction in Env-specific NK cell activation in 26/49 (53%) subjects. A 10-fold reduction in ADCC endpoint titers between baseline and week 96 was detected in both RFADCC and NK activation ADCC assay in the subset of samples tested. Serum Env-specific antibody binding titers significantly decreased 10-fold after 96 weeks of cART (baseline: median: 5 log10, IQR: 4.5-5; week 96: median: 4 log10, IQR: 3.5-4.5; p< 0.0001).

Conclusion: This longitudinal study showed a significant reduction in HIV-specific ADCC in HIV-infected subjects following cART. This may reduce the capacity for ADCC to clear or control reactivated latent HIV. ADCC-based therapeutic vaccines and/or modulation of ADCC effector functions could assist in the control of HIV and the ability to clear reactivated latently infected cells to cure HIV.

B58 – 5-drug HAART during Primary HIV Infection Leads to a Reduction of Proviral DNA Levels in Comparison to Levels Achievable during Chronic Infection

PE51

5-drug HAART during Primary HIV Infection Leads to a Reduction of Proviral DNA Levels in Comparison to Levels Achievable during Chronic Infection

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Background: The New Era Study is an ongoing, prospective 7-year clinical trial initiated in 2009 using multi drug class (MDC) HAART in patients (pts) with primary HIV infection (PHI, ≤2 Western blot bands) and in pts with chronic HIV-infection on suppressive PI-based HAART for ≥3 years without prior virologic failure (CHR). The primary objectives of the study were to halt residual viral replication in plasma and to achieve depletion of cell-associated HIV-DNA ('proviral DNA') as a step towards (functional) HIV cure.

Methods: Eligibility criteria were CD4 nadir >200/µl, no history of AIDS and CCR5 tropism. PHI pts received MDC HAART including 2 NRTIs +1 PI +Maraviroc (MVC) +Raltegravir (RAL). In CHR pts, HAART was intensified with MVC+RAL. HIV-DNA in peripheral blood mononuclear cells (PBMC) was measured as described by the French ANRS group. Here we compare virologic and immunologic outcomes after 24 months on MDC HAART in PHI and CHR pts.

Results: In total, 20 CHR and 22 PHI pts were included. PHI pts were started on MDC HAART within ≤2.6 weeks after diagnosis. Western blot was negative in 12 PHI pts. By month 24, cell-associated HIV-DNA had decreased significantly in PHI pts (median: -1.4 log cp/10^6 PBMC, p< 0.001) but not in CHR pts (median: +0.2 log cp/10^6 PBMC). At month 24, median proviral DNA levels were significantly lower in PHI pts (2.1 vs. 2.6 log cp/10^6 PBMC, p=0.001). The slopes of cell-associated-HIV-DNA are shown in the figure. After 24 months, significantly more PHI pts had a CD4/CD8-ratio ≥1 (90% vs. 35%, p=0.001). Proportions of activated CD38+ CD8+cells were comparable between groups (median levels: 13% vs. 13%, p=n.s.).

Conclusion: PHI patients receiving early treatment with multi drug class HAART achieved lower cell-associated HIV-DNA levels and a better immune reconstitution than chronically infected patients on intensified long-term suppressive HAART.

D24 – Impact of financial crises on the HIV funding and response

PE52

Building the Path to the End of AIDS: How investments in HIV prevention research and development responded to the global economic downturn, new research results and funding trends 2000-2012

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Background: Since 2004, the HIV Vaccines and Microbicides Resource Tracking Working Group has employed a comprehensive methodology to track trends in research and development (R&D) investments and expenditures for biomedical HIV prevention options, including HIV vaccines, microbicides, PrEP, treatment as prevention and adult voluntary medical male circumcision.

Methods: R&D data were collected on annual disbursements by public, private and philanthropic funders for product development, clinical trials and trial preparation, community education and policy advocacy efforts in order to estimate annual investment in HIV prevention R&D. Investment trends were assessed and compared by year, prevention technology type, funder category and geographic location.

Results: Five years ago, the global financial downturn was expected to have a significant and devastating impact on financial investments in R&D for AIDS. Pressure mounted to divert funding away from longer-term research in finding an HIV vaccine and effective microbicide towards proven technologies. In spite of this, overall investments in HIV prevention R&D have not decreased significantly, but instead virtually flatlined. Even as budgets tightened and donor capacity waned, science forged ahead, releasing groundbreaking results from microbicides, treatment as prevention, PrEP and HIV vaccine trials. While the promising results of recent years revitalized interest in advancing HIV prevention science, the effects of the 2008 financial crisis continued to be felt in 2012, as philanthropies developed more targeted investment strategies and public agencies funded fewer grants. Preliminary results show overall funding increased slightly in 2012, although some large donors decreased their investments.

Conclusion: In a financial climate with increasingly limited resources, tracking investment in HIV prevention R&D has continued to provide the field with vital information to chart the course forward, even in uncertain times. Monitoring funding trends better facilitates identification of promising areas where investment is needed, prioritization of research in those areas, assessment of the influence of public policies on funding trends and fact-based advocacy to support future research investments. As later-stage and follow-on trials advance, understanding and evaluating research in the volatile context of public, private and philanthropic funding is proving ever more important to ensuring progress down the path towards ending the AIDS pandemic.