The IAS would like to thank the following organizations for their generous support to the 2015 Towards an HIV Cure Symposium:
Contents

Oral Abstract Session 1:  5
Pathways to establishing and maintaining HIV latency
  OA1-1  5
  OA1-2  6
  OA1-3  7
  OA1-4 LB  8

Oral Abstract Session 2:  9
Activating latent HIV infection in vitro and in vivo
  OA2-1  9
  OA2-2  10
  OA2-3  11
  OA2-4  12
  OA2-5  13
  OA2-6 LB  14

Oral Abstract Session 3:  15
Novel strategies to identify and quantify virus persistence in vivo (biomarkers)
  OA3-1  15
  OA3-2  16
  OA3-3  17
  OA3-4  18
  OA3-5 LB  19

Oral Abstract Session 4:  20
Immunology and persistence
  OA4-1  20
  OA4-2  21
  OA4-3  22
  OA4-4 LB  23
  OA4-5 LB  24

Poster Exhibition
  Reverse transcription and integration  25
  Intrinsic cellular defences and restriction factors  27
  Type I Interferons (viral inhibition, immunomodulatory functions)  30
  Viral mechanisms of HIV/SIV persistence and latency  31
  Host cellular factors and latency  33
  Cellular and tissue reservoirs of HIV/SIV  34
Measurement of HIV/SIV reservoirs 38
HIV-1 controllers (including post-treatment controllers) 48
Asymptomatic long term non-progression 49
Targeting HIV persistence during ART (cure strategies) 51
Novel approaches in Immunotherapeutics (including bnAbs and anti-inflammatory mediators) 67
Therapeutic vaccines 71
Novel animal/virus models for vaccine, cure research, and inhibitor development 77
Acute and early infection 78
Long-term non-progressors and elite controllers 86
Timing of therapy initiation 88
Ethical issues in clinical trials and treatment strategies 91
Therapeutic vaccine trials 93
Complementary and traditional medicines 97
Curative interventions (including those aimed at reservoir depletion) 98
Novel therapeutic approaches (including gene therapy) 101
Clinical trials and antiretroviral therapy in children and adolescents 106
Engagement of community in service delivery 107
Oral Abstract Session 1: Pathways to establishing and maintaining HIV latency

OA1-1

CTLA-4-expressing memory CD4+ T-cells are critical contributors to SIV viral persistence

McGary C.¹, Paganini S.¹, Cervasi B.¹, Yu X.², Lichterfeld M.², Silvestri G.¹, Paiardini M.¹
¹Emory University, YNPRC, Atlanta, United States, ²Ragon Institute, Boston, United States

**Background:** Understanding the immunophenotype and anatomic location of latently infected cells represents a critical challenge in designing a cure for HIV. Among memory CD4+ T-cells, those expressing co-inhibitory receptors (Co-IRs) are strong candidates for being enriched in latent HIV, given their negative regulatory function and upregulation on T-cells following HIV infection. However, little is known regarding the dynamics of T-cells expressing multiple Co-IRs following suppressive ART and their contribution to the HIV/SIV reservoir, particularly in tissues.

**Methods:** We investigated the relationship between the level of Co-IR expression on memory CD4+ T-cells and their level of latent virus in 10 ART-treated, SIV-infected rhesus macaques (RMs). RMs initiated a 5-drug ART regimen 6-8 weeks after SIVmac251 infection, which was maintained until plasma viremia was < 60 copies/mL for at least 3 months. Blood and tissue levels of memory CD4+ T-cells expressing multiple Co-IR (PD-1, CTLA-4, TIM-3, 2B4, TIGIT) were longitudinally analyzed by flow cytometry. Memory CD4+Co-IR+ subsets were sorted twice during viral suppression based on their expression of PD-1, CTLA-4, and TIM-3, to quantify levels of cell-associated SIV-DNA and RNA.

**Results:** The majority of memory CD4+ T-cells from the blood, GI tract, lymph node, and spleen expressed multiple Co-IRs, specifically PD-1 and CTLA-4, and their frequencies remained stable or increased during SIV infection, even with suppressive ART. Following 1 month of viral suppression, both memory CTLA-4+(PD-1-) and PD-1+(CTLA-4-) CD4+ T-cells harbored significantly higher levels of SIV-DNA in the LN. Yet, after 3 months of suppression, only CTLA-4+ CD4+ T-cells, in the absence of other Co-IRs, were significantly enriched in SIV-DNA in the PBMCs, compared to Co-IR(-) cells, and in the LN, demonstrating the specific persistence of this virally infected subset. Furthermore, this subset did not express high levels of SIV-RNA, which suggests that these CTLA-4+ cells likely harbor latent SIV.

**Conclusions:** Despite comprising a small frequency of memory CD4+ T-cells, CTLA-4+ T-cells represent a novel subset of virally enriched cells that may critically contribute to persistence in ART-suppressed individuals. These findings highlight the benefit of therapeutically blocking both CTLA-4 and PD-1 to target a large fraction of the HIV reservoir.
OA1-2

Molecular determinants of HIV-1 permissiveness and persistence in gut-homing CD4+ T-cells expressing the Th17 marker CCR6

Planas D.1,2, Gosselin A.1, Monteiro P.1, Goulet J.-P.3, Da Fonseca S.1,2, Cléret-Buhot A.1,2, Jenabian M.-A.4, Routy J.-P.5,6, Ancuta P.1,2
1CHUM-Research Center, Montreal, Canada, 2University of Montreal, Microbiology Infectiology and Immunology, Montreal, Canada, 3CATRaGENE, Montreal, Canada, 4Université du Québec à Montréal (UQAM), Department of Biological Sciences and BioMed Research Centre, Montreal, Canada, 5McGill University Health Center, Division of Hematology, Montreal, Canada, 6McGill University Health Center, Immunodeficiency Service, Montreal, Canada

Background: HIV-infected CD4+ T-cells are enriched in gut-associated lymphoid tissues (GALT). The integrin α4β7 and CCR9 mediate imprinting for gut-homing, and their expression is induced by retinoic acid (RA), a vitamin A metabolite produced by GALT dendritic cells. We previously demonstrated that CD4+ T-cells expressing the Th17 marker CCR6 are permissive to HIV in vitro, harbor replication-competent HIV reservoirs in ART-treated subjects, and that RA selectively increases HIV replication in these cells. To identify new molecular determinants of HIV permissiveness/persistence, we performed a genome-wide transcriptional analysis in RA-treated CCR6+ versus CCR6- T-cells.

Methods: CD4+ T-cells were sorted from PBMCs by negative selection using magnetic beads (Miltenyi). Memory (CD45RA-) CCR6+ and CCR6- T-cells were sorted by flow cytometry (BDariaII). Cells were stimulated via CD3/CD28 and cultivated in the presence or absence of RA (10nM) for 4 days. Total RNA was extracted for microarrays analysis (HT 12v4 BeadChip, Illumina; >46,000 probe sets per chip). Validations of microarrays were performed by real-time PCR and/or flow cytometry. HIV-DNA integration was measured by nested real-time PCR. Functional validations were performed using RNA interference (Amaxa).

Results: Among 15,303 “present calls”, 1,538 and 1,285 probe sets were modulated by RA in CCR6- and CCR6+ T-cells, respectively (p-value < 0.05; fold change cut-off 1.3). Gene Set Variation Analysis (GSVA), Ingenuity Pathway Analysis (IPA), and Gene Ontology tools were used to identify pathways/individual transcripts specifically induced by RA in CCR6+ versus CCR6- T-cells. This signature included an increased expression of gut homing markers (α4β7, CCR9), HIV-1 coreceptors (CCR5, CXCR6), and also pathways linked to the regulation of T-cell activation (CD38, Lck, PTPN13, MAP4K4), glucose metabolism (Glut1, Glut8), cell cycle (GADD45G), HIV replication via CCR5 expression (KLF2), and multidrug resistance (MDR1/ABCB1). In addition, the transcriptome of RA-treated CCR6+ T-cells showed decreased expression of known HIV-1 resistance factors (PPAR-g, CCL3, CCL3L1).

Conclusions: Our studies demonstrate that RA-mediated imprinting for gut-homing is associated with HIV permissiveness in CCR6+ but not CCR6- T-cells and reveal molecular mechanisms underlying these differences. These findings will orient the discovery of new therapeutic strategies aimed at limiting HIV permissiveness, and subsequently the size of HIV reservoirs, specifically in gut-homing Th17 cells.

Under embargo until 14.30 on 22 July 2015
Dasatinib preserves SAMHD1 antiviral activity in CD4+ T-cells treated with IL-7

Alcami J., Bermejo M., Descours B., Mateos E., Lederman M.M., Benkirane M., Coiras M.

1Instituto de Salud Carlos III, Microbiology, Majadahonda, Spain, 2Institute of Human Genetics, Montpellier, France, 3Case Western Reserve University School of Medicine, Cleveland, United States

Background: HIV-1 post-integration latency in quiescent CD4+ T cells is responsible for viral persistence despite antiretroviral treatment. It was proposed that the increase in proviral load in HIV-infected patients after IL-7 treatment was due to homeostatic proliferation of memory CD4+ T cells. We determined previously that IL-7 increased HIV-1 infection through phosphorylation and subsequent inactivation of the restriction factor SAMHD1. Now we analysed SAMHD1 phosphorylation in PBMC from patients enrolled in ACTG 5214 study (NTC00099671), in order to elucidate the role of IL-7 in HIV-1 proviral integration and persistence and whether this could be related to SAMHD1 inactivation. In addition, we determined that the tyrosine-kinase inhibitor Dasatinib preserved SAMHD1 antiviral activity, avoiding IL-7-mediated HIV-1 infection.

Methods: PBMC samples obtained from 10 patients enrolled in ACTG 5214 study (NTC00099671), collected before (day 0) and 4 after administration of IL-7. PBMCs obtained from 2 patients diagnosed with chronic myeloid leukemia (CML), on chronic treatment with Dasatinib. Resting CD4+ T cells from healthy donors obtained by negative selection from PBMCs. Phosphorylation of SAMHD1 at T592 was determined by immunoblotting and flow cytometry. Proviral integration was analyzed by TaqMan qPCR. Dasatinib (BMS-354825, Sprycel) was provided by Bristol-Meyers Squibb.

Results: 1) IL-7 (1nM) induced SAMHD1 phosphorylation, interfering with its antiviral activity. 2) IL-7-mediated SAMHD1 phosphorylation greatly increased HIV-1 infection in purified CD4+ T cells, increasing early and late retrotranscription, as well as proviral integration. 3) A significant increase in pSAMHD1 was observed in central memory CD4+ T cells from HIV-infected patients treated with IL-7 (ACTG 5214). 4) Dasatinib completely inhibited SAMHD1 phosphorylation at 75nM, interfering with HIV-1 retrotranscription and consequently, with proviral integration. 5) CD4+ T cells from patients with CML treated with Dasatinib showed lower expression of SAMHD1 phosphorylated.

Conclusions: By inducing SAMHD1 phosphorylation, IL-7 increases susceptibility of resting CD4+ T lymphocytes to infection, leading to HIV persistence. SAMHD1 regulation plays a central role in the establishment of HIV-1 reservoirs and represents a major target for therapeutic intervention. Dasatinib is the first compound currently used in clinic that has been described to preserve the antiviral function of an innate factor such as SAMHD1.
OA1-4 LB

Estrogen blocks HIV re-emergence from latency and points to gender-specific differences in HIV reservoirs

J. Karn1, B. Das1, C. Dobrowolski1, E. Scully2, S. Deeks1, M. Gandhi3, R. Johnston4

1Case Western Reserve University School of Medicine, Department of Molecular Biology and Microbiology, Cleveland, United States, 2Brigham and Women's/Massachusetts General Hospital, Boston, United States, 3UCSF School of Medicine, San Francisco, United States, 4amfAR, The Foundation for AIDS Research, New York, United States

Background: Unbiased shRNA library screens have been used to identify novel genes and pathways that are required to maintain HIV latency and/or play an essential role in HIV transcription. One of the most prominent and robust “hits” was the estrogen receptor type 1 (ESR-1).

Methods: The activities of ESR-1 agonists, antagonists and estrogen on proviral reactivation were studied in transformed and primary cell models of latency and in patient cells.

Results: specific antagonists of ESR-1, such as Tamoxifen and Fulvestrant, are weak proviral activators but sensitize latently infected cells to very low doses of the proviral activators TNF-α (NF-κB inducer) and SAHA (HDAC inhibitor). By contrast, a selective ESR-1 agonist, propylpyrazoletriol (PPT) and the broader spectrum ESR-1 agonist diethylstilbestrol, strongly suppress both TNF-α and SAHA reactivation. In contrast to the ESR-1 antagonists, ESR-2 antagonists were not effective inducers of HIV expression in cell models. Co-activator 3 (SRC-3) is an upstream modulator of ESR-1, which also was identified as a hit in the shRNA screen. Blocking of SRC-3 by its inhibitor Gossypol also induces latent proviruses. Consistent with these results, specific knock-down of ESR-1 in Jurkat 2D10 cells with shRNA constitutively re-activates the latent provirus. In the HAART-treated patient samples there was a modest increase of spliced HIV env mRNA when resting memory cells were treated with the ESR antagonists Fulvestrant or Tamoxifen alone. Proviral reactivation by ESR antagonists was synergistically increased by SAHA. By contrast, β-Estradiol at concentrations in the physiological range led to dramatic reductions in proviral reactivation efficiencies. This is consistent with earlier observations that high levels of β-Estradiol can block HIV replication.

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

Under embargo until 14.30 on 21 July 2015
OA2-1

Histone deacetylase inhibitors alter the accumulation of spliced HIV mRNA - implications for virus production

Mota T.M.¹,², Wightman F.²,³, Cheong K.Y.², Lu H.K.², Anderson J.L.²,³, Solomon A.²,³, Purcell D.F.¹,², Lewin S.R.²,³
¹University of Melbourne, Department of Microbiology and Immunology, Melbourne, Australia, ²University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, Australia, ³Alfred Hospital and Monash University, Department of Infectious Diseases, Melbourne, Australia

Background: Clinical trials in HIV-infected patients on antiretroviral therapy with histone deacetylase inhibitors (HDACi) have demonstrated an increase in cell-associated unspliced (CA-US) HIV RNA, variable changes in plasma HIV RNA and no change in the number of latently infected cells. We aimed to define the effects of latency reversing agents (LRAs) on HIV mRNA splicing.

Methods: Resting CD4+ T cells isolated from the blood of HIV-negative individuals were treated with the chemokine CCL19 and infected with wild type HIVNL4.3 to establish latency (n=5). Latently infected CCL19-stimulated cells were then cultured with vorinostat, romidepsin, JQ1, romidepsin+JQ1 or PMA/PHA, all in the presence of an integrase inhibitor (L8). Cells and supernatant were harvested at 6, 24, 48, and 72 hours. Reverse transcriptase (RT) was quantified in supernatant and CA-US and multiply spliced (MS) HIV RNA were quantified by real time qPCR.

Results: In latently infected CCL19-treated CD4+ T-cells, stimulation with PMA/PHA led to a significant exponential increase in both US-RNA and MS-RNA, and by 48 hours reached a mean fold increase above baseline of 80-fold for US-RNA and 56-fold for MS-RNA (p=0.03 for both, relative to DMSO). There was a significant increase in RT in supernatant following stimulation with PMA/PHA but no change following any LRA (n=2). In contrast, following stimulation with each LRA, there was only a modest increase in CA-US RNA that was not statistically significantly different from DMSO (p=0.56). MS-RNA increased transiently (mean 2.7-fold change at 6hr with romidepsin) and then significantly declined over time following treatment with romidepsin and romidepsin+JQ1 (p=0.02 and 0.002 respectively), with a mean fold reduction by 72 hours compared to baseline of 0.15-fold and 0.17-fold respectively (p=0.02 for both compared to DMSO) in the absence of any cellular cytotoxicity.

Conclusions: In this in vitro model of latency, PMA/PHA and the potent HDACi romidepsin had strikingly different effects on the accumulation of US-RNA, MS-RNA and virus production. While successful HDACi agents yield small increases in US-RNA, synergistic strategies that achieve a larger accumulation of MS RNA may result in enhanced release of latent HIV.
Latency reversal Agent (LRA) romidepsin reactivates latent virus in two rhesus macaque (RM) models of controlled SIV infection in the absence of antiretroviral therapy (ART)

Policicchio B.\textsuperscript{1,2}, Brocca-Cofano E.\textsuperscript{1}, Xu C.\textsuperscript{1}, Ma D.\textsuperscript{1}, He T.\textsuperscript{1,3}, Li H.\textsuperscript{4}, Haret-Richter G.\textsuperscript{1}, Dunsmore T.\textsuperscript{1}, Shaw G.\textsuperscript{4}, Ribeiro R.\textsuperscript{5}, Pandrea I.\textsuperscript{1,3}, Apetrei C.\textsuperscript{1,6}

\textsuperscript{1}University of Pittsburgh, Center for Vaccine Research, Pittsburgh, United States, \textsuperscript{2}University of Pittsburgh, Graduate School of Public Health, Infectious Diseases and Microbiology, Pittsburgh, United States, \textsuperscript{3}University of Pittsburgh, School of Medicine, Cellular and Molecular Pathology, Pittsburgh, United States, \textsuperscript{4}University of Pennsylvania, School of Medicine, Philadelphia, United States, \textsuperscript{5}Los Alamos National Laboratory, Los Alamos, United States, \textsuperscript{6}University of Pittsburgh, School of Medicine, Microbiology and Molecular Genetics, Pittsburgh, United States

Background: Viral reservoirs represent a major obstacle for HIV cure research. A reservoir reactivation strategy is the “flush and kill”, in which LRAs reactivate latent virus and CTLs eliminate it. LRAs have limited efficacy, while immunosuppression impairs CTL ability to eliminate reactivated virus. Our goals were to assess in vivo romidepsin ability to reactivate SIV in two different models of controller RMs with functional immune responses and its effect on CTLs and viral control.

Methods: Three SIVsmmFTq-infected RMs received ART (PMPA; FTC; L-870812) for 9 months. After treatment discontinuation, the RMs controlled virus rebound and received 3 rounds of romidepsin, followed by CD8\textsuperscript{+} cell depletion. Two SIVsab-infected RM spontaneous controllers received two rounds of romidepsin. Plasma viral loads were monitored with single copy assays. PBMC histone acetylation, IFN-\(\gamma\) production by CTLs and changes in T cells counts and their immune activation/proliferation status were assessed by flow cytometry. Romidepsin toxicity was monitored clinically and biologically; T-cell apoptosis post-RMD was assessed flow-cytometrically and by LDH ELISA.

Results: Romidepsin administration resulted in significant virus rebounds (up to 104 copies/ml for SIVsmmFTq and 103 copies/ml for SIVsab) followed by gradual viral decline. Romidepsin was well-tolerated and induced a massive surge in T-cell activation and transient lymphopenia during the first week post-treatment. Lymphopenia resulted from cell redistribution and downregulation of surface markers rather than T-cell destruction. CD8\textsuperscript{+} cell depletion resulted in robust viral rebound (up to 107 copies/ml) that was controlled upon CD8\textsuperscript{+} T-cell recovery. Romidepsin did not significantly affect CTL antiviral functions in vivo. Using mathematical modeling, we showed that a small fraction of latently infected cells were at the origin of virus rebound.

Conclusions: Using two different in vivo models of SIV control, we demonstrated romidepsin can reactivate the reservoir virus. The levels of virus replication, timing of virus rebound and rapid control of virus replication after romidepsin administration suggest the reactivated virus is replication-competent and romidepsin does not persistently alter CTL function. CD8\textsuperscript{+} cell depletion resulted in higher viral rebound compared to romidepsin administration, suggesting that romidepsin does not completely ablate CTL function. Altogether, our results show romidepsin can effectively reverse SIV latency.
Vorinostat, panobinostat and romidepsin nonselectively activate transcription from quiescent HIV-1 proviruses in HIV-infected individuals on long-term suppressive anti-retroviral therapy

K. Barton1, T.A. Rasmussen2, M. Tolstrup2, W. Shao3, B. Hiener1, R. Olesen2, A. Winckelmann1, A. Solomon5,6,7, L. Østergaard2, S.R. Lewin5,6,7, O.S. Søgaard2, S. Palmer1

1University of Sydney, Westmead Millennium Institute, Westmead, Australia, 2Aarhus University Hospital, Department of Infectious Diseases, Aarhus, Denmark, 3Leidos Biomedical Research, Inc, Advanced Biomedical Computing Center, Reston, United States, 4Frederick National Laboratory for Cancer Research, Frederick, United States, 5Monash University, Department of Infectious Diseases, Alfred Hospital, Melbourne, Australia, 6Burnet Institute, Centre for Biomedical Research, Melbourne, Australia, 7The University of Melbourne, Doherty Institute for Infection and Immunity, Melbourne, Australia

Background: Clinical trials in HIV-infected individuals on long-term anti-retroviral therapy (ART) using histone deacetylase inhibitors (HDACis) to reverse HIV-1 latency have demonstrated a measurable increase in HIV-1 transcription in CD4 T cells in blood. However, for effective viral clearance, it is important that these compounds activate transcription from a broad range of integrated proviruses. In this study, we used sequencing to determine whether vorinostat, panobinostat, and romidepsin selectively or nonselectively target HIV-1 proviruses.

Methods: CD4 T cells were obtained from 36 participants before, during, and after HDACi treatment using vorinostat (n=15), panobinostat (n=15), and romidepsin (n=6). We used single-proviral/genome sequencing to characterize the genetic composition of the env region of cell-associated HIV-1 DNA and RNA to determine which HIV-1 proviruses were being transcribed in response to HDACi therapy within CD4 T cells. Additionally, for the panobinostat trial, we sequenced plasma HIV-1 RNA from samples collected during a post-HDACi ART interruption. Maximum-likelihood trees were constructed for each participant and the average-pairwise distance of the sequences was calculated using MEGA 6.0.

Results: The average-pairwise distance of the cell-associated HIV-1 RNA that was detected following administration of the HDACis was not significantly different from that of the cell-associated HIV-1 DNA (2.9% vs. 3.1%, p=0.79). Furthermore, upon phylogenetic analysis, the HIV-1 RNA sequences intermingled with the HIV-1 DNA sequences throughout the phylogenetic trees, supporting a broad and nonselective activation of HIV-1 proviruses. The plasma-derived sequences from the ART interruption samples contained expansions of identical sequences, which in three cases were identical to cell-associated DNA sequences. Additionally, cell-associated HIV-1 RNA had a significantly higher percentage of dead-end virus (hypermutated and/or containing stop codons) than the cell-associated HIV-1 DNA (40.1% vs. 7.8%, p=0.0004).

Conclusions: We found that vorinostat, panobinostat, and romidepsin nonselectively induce transcription from HIV-1 proviruses in HIV-infected individuals on long-term suppressive therapy, which is promising for the development of future therapies that aim to activate quiescent HIV-1 proviruses as part of an eradication strategy. Although, a large amount of cell-associated HIV-1 RNA was replication incompetent, we did identify cell-associated HIV-1 DNA that contributed to rebound virus during a post-HDACi ART interruption.

Under embargo until 14.30 on 21 July 2015
Modulation of HERV family expression after treatment with HDAC inhibitors

de Mulder Rougvie M.1, Brailey P.1, Saraiva Raposo R.A.1, Sengupta D.2, Michaud H.A.3, Leal F.1, Ormsby C.E.4, Jones R.B.5, Ostrowski M.6, Nixon D.F.1

1George Washington University, Microbiology, Immunology and Tropical Medicine, Washington, United States, 2University of California San Francisco, Department of Experimental Medicine, San Francisco, United States, 3Institut de Recherche en Cancérologie de Montpellier, Equipe Immunité et Cancer, Montpellier, France, 4Instituto Nacional de Enfermedades Respiratorias, Centro de Investigaciones en Enfermedades Infecciosas, Mexico City, Mexico, 5Massachusetts Institute of Technology and Harvard University, Ragon Institute, Boston, United States, 6University of Toronto, Department of Immunology, Toronto, Canada

Background: Human Endogenous Retroviruses (HERVs) comprise about 8% of the human genome. Some autoimmune diseases and cancers have been associated with the expression of HERV-K, which is the most recently integrated family of endogenous retroviruses. The production of HERV-K derived proteins in HIV infected cells provides a potential target for HIV eradication. Latently HIV infected remain as the major obstacle for HIV eradication. Use of histone deacetylase inhibitors (HDACis) to induce HIV expression in resting cells is a promising strategy for HIV latency reversal.

Methods: In this study we quantified the reactivation of five different families of HERVs by three non-selective HDACis (Vorinostat, Panobinostat and Romidepsin) in a latently HIV-1 T-cell model.

Results: After a 5-hour pulse with each HDACis, Vorinostat (1000nM), Panobinostat (50nM) and Romidepsin (50nM), we detected a 23.8%, 32.1% and 58.9% reactivation of HIV-1, respectively by measuring intracellular KC57 expression by flow cytometry. We also detected an increase in the gene expression of tested HERV families (R, K, H and P), with Panobinostat having the strongest ability to induce expression HERV-K. Further analysis within the HERV-K family, revealed that the pol gene was the most expressed gene compared to gag and env.

Conclusions: These data demonstrate the dynamic regulation of HERV expression after treatment with HDACis and future HIV-1 therapeutic strategies should consider the influence of the reactivation of endogenous retroviruses in infected cells.
OA2-5

Ingenol efficiently reactivates latent HIV in cells from aviremic patients

A. Spivak¹, A. Bosque¹, A. Balch³, V. Planelles²

¹University of Utah, Medicine, Salt Lake City, United States, ²University of Utah, Pathology, Salt Lake City, United States, ³University of Utah, Pediatrics, Salt Lake City, United States

Background: The HIV-1 latent reservoir represents a major barrier to viral eradication in aviremic HIV-1+ patients taking antiretroviral therapy (ART). Here, we describe a careful characterization of the promising LRA properties of Ingenol dibenzoate, panobinostat, and bromosporin, in cells from aviremic patients, including the effects on cellular activation. To accomplish this, we utilized a new-generation, rapid assay performed ex vivo with patient cells.

Methods: We formulated a rapid ex vivo assay using cells from healthy, aviremic HIV-1+ volunteers on stable ART. After phlebotomy, resting CD4+ T cells were isolated via negative magnetic bead purification and cultured in aliquots of 5x10⁶ cells / 1mL RPMI-based culture media. Cell aliquots were exposed to media alone (negative control), individual LRAs at concentrations previously demonstrated to induce viral reactivation, combinations of LRAs with unique mechanisms of action or antibodies against CD3 and CD28 to induce T cell receptor stimulation and cellular activation (positive control). After 48 hours in culture, quantitative rtPCR was performed using both culture supernatant and cell-associated RNA to detect HIV-1 viral mRNA. Cryopreserved cell aliquots from each condition were evaluated by flow cytometry for biomarkers of drug activity, cellular activation and toxicity.

Results: Ingenol 3,20-dibenzoate, a PKCα agonist, demonstrated viral reactivation comparable to CD3/28 antibody stimulation (median reactivation = 49% of positive control). CD69, an early marker of T cell activation known to be up-regulated by ingenol, increased in all cell aliquots exposed to ingenol (MFI = 81% of positive control). Bromosporin, a BRD inhibitor, and Panobinostat, an HDAC inhibitor, demonstrated modest activity in a subset of patients.

Conclusions: Because of the diversity of cell culture models of latency and the lack of uniformity in the responses across models, we made an effort to develop a rapid ex vivo assay using cells from aviremic HIV-1+ patients to confirm bioactivity and characterize latency-reversing potential of candidate LRAs individually and in combination. This assay was used to characterize the activity of Ingenol as an exciting LRA candidate, as it combines a potent reactivation ability with a very low toxicity profile, which sets it apart from other PKC agonists that are, unfortunately highly toxic.
CD4 mimetics sensitize HIV-1-infected cells to ADCC

J. Richard1,2, M. Veillette1,2, N. Brassard1, S.S. Iye1, M. Roger1,2, L. Martin1, M. Pazgier5, A. Schön6, E. Freire6, J.-P. Routy7, A.B. Smith rd1,2, J. Park3, D.M. Jones3, J.R. Courter3, B.N. Melillo3, D.E. Kaufmann1,2,8, B.H. Hahn3, S. Permarl9, B.F. Haynes9, N. Madani10,11, J. Sodroski10,11, A. Finzi1,2,7

1The CHUM Research Center, Montreal, Canada, 2University of Montreal, Montreal, Canada, 3University of Pennsylvania, Philadelphia, United States, 4dCEA, iBiTecS, Service d’Ingénierie Moléculaire des Protéines, Gif sur Yvette, France, 5University of Maryland School of Medicine, Baltimore, United States, 6The Johns Hopkins University, Baltimore, United States, 7McGill University, Montreal, Canada, 8Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard, Cambridge, United States, 9Duke Human Vaccine Institute, Duke University Medical Center, Duke, United States, 10Dana-Farber Cancer Institute, Boston, United States, 11Harvard School of Public Health, Boston, United States

**Background:** Prevention of HIV-1 transmission and progression likely requires approaches that can specifically eliminate HIV-1-infected cells. There is increasing evidence supporting a role of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) in controlling HIV-1 transmission and disease progression. Importantly, the interaction of HIV-1 envelope (Env) glycoproteins with the CD4 receptor was recently reported to be required for efficient exposure of ADCC-mediating Env epitopes. In that context, HIV-1-infected cells presenting HIV-1 Env in the CD4-bound conformation on their surface were found to be preferentially targeted by ADCC-mediating antibodies present in sera of HIV-1-infected individuals. However, HIV-1 has evolved a sophisticated mechanism to avoid exposure of ADCC-mediating Env epitopes by downregulating CD4 and by limiting the overall amount of Env at the cell surface.

**Methods:** Rationally-designed CD4-mimetic compounds (CD4mc) have been shown to induce thermodynamic changes in HIV-1 Env similar to those induced by CD4 and sensitize HIV-1 particles to neutralization by otherwise non-neutralizing CD4-induced antibodies. In this study, we explored the capacity of such compounds to promote the CD4-bound conformation of Env and thereby sensitize HIV-1-infected cells to ADCC mediated by sera, cervico-vaginal lavages and breast milk from HIV-1-infected individuals. However, HIV-1 has evolved a sophisticated mechanism to avoid exposure of ADCC-mediating Env epitopes by downregulating CD4 and by limiting the overall amount of Env at the cell surface.

**Results:** We observed that certain CD4mc induce the CD4-bound conformation of Env and thereby sensitize cells infected with primary HIV-1 isolates to ADCC mediated by prevalent and easy-to-elicit antibodies present in sera from early converters and chronically-infected individuals. Importantly, CD4mc also enhanced recognition and ADCC-mediated elimination of HIV-1-infected cells by antibodies present in breast milk and cervico-vaginal lavages of HIV-1-infected women. Finally, we identified one CD4mc with the capacity to sensitize endogenously-infected ex-vivo-amplified primary CD4 T cells to ADCC killing mediated by autologous sera and effector cells.

**Conclusions:** By pushing Env into the CD4-bound conformation, CD4mc might represent an alternative and/or complementary approach to currently-available drugs for preventing viral transmission and might represent a new strategy aimed at eradicating the viral reservoir.
OA3-1

Immunological markers associated with HIV persistence during ART identified by iterated conditional random forests analysis

Fromentin R.1, Ramirez C.M.2, Khoury G.1, Sinclair E.1, Hecht F.M.4, Deeks S.G.4, Sekaly R.P.5, Lewin S.R.3,6, Chomont N.1,7

1CRCHUM, Montreal, Canada, 2University of California Los Angeles, Department of Biostatistics, Los Angeles, United States, 3Alfred Hospital and Monash University, Department of Infectious Diseases, Melbourne, Australia, 4University of California San Francisco, Department of Medicine, San Francisco, United States, 5Case Western Reserve University, Department of Pathology, Cleveland, United States, 6The University of Melbourne, Doherty Institute for Infection and Immunity, Melbourne, Australia, 7Université de Montréal, Department of Microbiology, Infectiology and Immunology, Montreal, Canada

Background: The persistence of latently infected cells and residual levels of viral production contribute to HIV persistence and immune activation in HIV infected individuals on suppressive antiretroviral therapy (ART) and represent major barriers to HIV eradication. We hypothesized that HIV persistence on ART was associated with markers of T-cell activation, homing and proliferation.

Methods: Expression of activation/proliferation markers, chemokines receptors, immune checkpoints and their ligands were measured by flow cytometry on PBMCs isolated from 48 HIV-infected subjects on ART for >3 years with HIV viral load < 50 copies/ml and with a CD4 count >350 cells/µL. Two virological markers of HIV persistence were determined by quantitative (q)PCR: the frequencies of CD4 T cells harboring integrated HIV DNA and cell associated unspliced (CA-US) HIV RNA. Chemokines, gamma-c cytokines and sCD14 were quantified in plasma. More than 600 variables were analyzed by fuzzy forests to identify novel biomarkers associated with HIV persistence and that predict low reservoir size. Briefly fuzzy forests first separates the variables into modules that have a similar correlation structure to account for network effects and then performs recursive feature elimination random forests to find the top parameters that are predictive of the outcome.

Results: Using fuzzy forests, we identified the top 100 variables of importance/predictors that were most strongly associated with high frequency of CD4 T cells harboring integrated HIV DNA or CA-US HIV RNA. High CA-US RNA was strongly associated with activating IFN signaling pathway in T cells (pSTAT1-3). High frequency of cells harboring integrated HIV DNA was associated with low CD4 count (p=0.0015) as expected but also with higher frequency of cells expressing markers of proliferation/activation (including expression of 2B4, LAG3, TIGIT on central memory CD4 T cells, p=0.0057, p=0.0016, p=0.0106 respectively and HLA-DR and CD38 on CD8 T cells, p=0.019 and p=0.0252 respectively).

Conclusions: Current assays that measure virus persistence are associated with different immunological pathways. CA-US RNA, a surrogate marker of active viral transcription, was associated with the STAT1-3 downstream of type I interferon signaling pathway while the number of latently infected cells was associated with markers of T-cell activation, proliferation and exhaustion.
OA3-2

Patient-derived defective HIV-1 proviruses containing large internal deletions can be transcribed and translated

Pollack R.1, Bruner K.1, Ho Y.-C.1, Siliciano R.1,2
1Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore MD, 2Howard Hughes Medical Institute, Baltimore MD

Background: Despite antiretroviral therapy (ART), human immunodeficiency virus-1 (HIV-1) persists as integrated proviruses primarily in resting CD4+ T cells as the major barrier to cure. The majority of these proviruses are defective, containing large internal deletions or APOBEC-mediated G-to-A hypermutations. With intact promoter function and lack of epigenetic silencing, some defective proviruses can potentially be transcribed. Transcription of defective proviruses may complicate the measurement of latency reversal using PCR-based assays, such as cell-associated RNA quantification. We have previously reported that hypermutated proviruses can be transcribed from patient resting CD4+ T cells. However, it remains unclear whether proviruses containing large internal deletions can be transcribed. Identification of the proviral components (single genes such as tat and rev or specific region of the proviral genome) required for defective proviruses to be transcribed may help to design a more accurate assay for the measurement of latency reversal.

Methods: To identify the proviral genes essential for viral transcription, we constructed proviral clones defective in gag, tat and/or rev by site-directed mutagenesis of the NL4-3 reference strain. To identify the type of defective provirus which can be transcribed, we reconstructed full-length defective proviral clones containing large internal deletions (encompassing the packaging signal, 5' or 3' of the proviral genome) and hypermutations. These clones were isolated from resting CD4+ T cells of HIV-1-infected individuals under suppressive ART through limiting-dilution PCR and reconstructed through de novo full-length genome synthesis. Primary CD4+ T cells were transfected with proviruses and activated by CD3/CD28 co-stimulation. After DNase treatment, cell-associated HIV-1 RNA was measured by quantitative RT-PCR. HIV-1 protein expression was measured by flow cytometry of the Gag protein.

Results: We reconstructed 11 full-length defective proviral clones from HIV-1-infected individuals. Defective proviruses with intact tat genes can produce HIV-1 RNA at lower but significant levels following CD3/CD28 co-stimulation. These defective proviruses are capable of producing HIV-1 viral proteins at measurable but lower levels than the NL4-3 reference strain.

Conclusions: Defective HIV-1 proviruses can be transcribed and translated following stimulation. HIV-1 transcription from defective proviruses, especially those containing an intact tat, should be considered in the measurement of latency reversal.

Under embargo until 11.00 on 20 July 2015
OA3-3

Cell-associated HIV-1 unspliced to multiply spliced RNA ratio at 12 weeks ART correlates with markers of immune activation and apoptosis and predicts the CD4+ T-cell count at 96 weeks ART

Pasternak A., Scherpenisse M., Berkhout B.
Academic Medical Center of the University of Amsterdam, Medical Microbiology, Amsterdam, Netherlands

Background: Incomplete restoration of CD4+ T-cell count during virologically successful antiretroviral therapy (ART) is a major predictor of morbidity and mortality. For better understanding of HIV-1 pathogenesis and improved design of curative strategies, it is important to determine whether the degree of HIV-1 persistence, measured at baseline or early on ART, can predict subsequent immunological response to the long-term therapy and whether viral persistence is associated with host biomarkers of immune dysfunction.

Methods: Total and episomal (2-LTR circles) HIV-1 DNA, unspliced and multiply spliced (total and tat/rev) cell-associated HIV-1 RNA, as well as markers of CD4+ and CD8+ T-cell activation, proliferation, senescence, apoptosis, exhaustion, thymic migration, and CD4+ and CD8+ T-cell subsets (naïve, central memory, effector memory, transitional memory), were longitudinally measured in a cohort of 28 HIV-infected patients at 0, 12, 24, 48, and 96 weeks of virologically suppressive ART.

Results: No baseline HIV-1 marker was predictive of CD4+ T-cell count at 96 weeks of ART. However, at 12 weeks of ART, cell-associated HIV-1 unspliced to multiply spliced-total (US/MS) RNA ratio strongly negatively correlated with both absolute CD4+ T-cell count at 96 weeks of ART (rho=-0.56, P=0.004) and with relative increase in CD4+ T-cell count between baseline and 96 weeks of ART (rho=-0.55, P=0.004). US/MS RNA ratio at 12 weeks ART was not associated with baseline CD4+ T-cell count. Moreover, US/MS RNA ratio at 12 weeks ART strongly positively correlated with markers of CD4+ T-cell activation (CD4+/CD38+/HLA-DR+: rho=0.63, P=0.001) and apoptosis (CD4+/Annexin-V+/FAS+: rho=0.59, P=0.002).

Conclusions: We observed that US/MS RNA ratio at 12 weeks ART positively correlated with immune activation and apoptosis and predicted lower CD4+ T-cell count at 96 weeks ART. Because HIV life cycle involves a temporal shift from the production of multiply spliced to the production of unspliced RNA species, higher US/MS RNA ratio in a patient might reflect the higher frequency of HIV-infected cells in the later stages of viral life cycle, which is characterized by expression of viral proteins and presentation of antigens. Such cells could exert pressure on the host immune system, causing persistent immune activation and apoptosis and contributing to poor immunological response to ART.
OA3-4

Distinct HIV genetic populations in effector memory T-cells after prolonged therapy

Lee E,1,2, Hiener B,1,2, Bacchetti P,1, Shao W,1, Boritz E,3, Douek D,1, Somsouk M,6, Hunt P,6, Fromentin R,7, Deeks S.G,6, Hecht F.M,6, Chomont N,7, Palmer S,1,2

1Westmead Millennium Institute for Medical Research, Centre for Virus Research, Westmead, Australia, 2University of Sydney, Sydney Medical School, Sydney, Australia, 3University of California San Francisco, Department of Epidemiology and Biostatistics, San Francisco, United States, 4Leidos Biomedical Research, INC, Frederick National Laboratory for Cancer Research, Frederick, United States, 5National Institutes of Health, Immunology Laboratory, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, United States, 6University of California San Francisco, Department of Medicine, San Francisco, United States, 7Vaccine and Gene Therapy Institute of Florida, Port Saint Lucie, United States

Background: The effect of prolonged antiretroviral therapy (ART) on the genetic composition of persistent HIV in cellular reservoirs is unknown. We examined the genetic makeup of HIV DNA sequences within T-cell subsets from peripheral blood and gut tissue of persons on ART for >15 years.

Methods: Using single-proviral sequencing, we isolated HIV DNA from naïve, stem cell memory (TSCM), central (TCM)-, transitional (TTM)- and effector (TEM)-memory and homing CD4+ T-cells (expressing CCR6, CXCR5 or both markers) sorted from peripheral blood and total CD4+ T-cells sorted from rectal biopsies. Samples were collected from 6 subjects on ART for >15 years: 3 who initiated therapy during early infection and 3 during chronic infection. Hypermutants, drug resistance mutations and identical sequences were identified by phylogenetic analysis. We used the tat/rev induced limiting dilution assay (TILDA) to measure the frequency of cells with inducible multiply spliced HIV RNAs (msRNAs).

Results: In subjects treated during chronic infection, TEM contained genetically distinct HIV populations, often clonal in nature, compared to other cells. In one subject all HIV sequences (n=62) from TEM were hypermutants and 82% clonal, whereas all other T-cell subsets had significantly fewer identical HIV DNA hypermutants (p=< 0.0001-0.001). Another subject, with a history of sequential ART regimens, had wildtype HIV sequences in TEM (92%) and more drug resistant HIV in other T-cell subsets (p=< 0.0001-0.0004). TEM from the third chronic subject contained 73% clonal drug resistant HIV sequences whereas the other cells had only 7-15% (p=< 0.0001-0.0002). In one subject treated during early infection, 44% of all HIV sequences were hypermutant: 56% in TEM and 86% in CD4+ T-cells from rectal biopsies. All subjects had inducible HIV msRNAs in memory T-cell subsets as measured by TILDA but msRNAs were lower in TEM from individuals containing hypermutant populations.

Conclusions: The distribution of HIV genetic material among memory subsets varied dramatically across the cohort after prolonged ART. TEM are marked by clonal expansions which may reflect random antigen-driven cellular proliferation and expansion. Enrichment of hypermutant HIV in TEM suggests new infection events during proliferative bursts are attenuated by cellular restriction factors and/or by death of cells expressing replication competent virus.
Investigating the role of the immune checkpoint receptor TIGIT in T cells during HIV disease progression and as a target for immune restoration


1University of Hawaii, Manoa, Department of Tropical Medicine, Medical Microbiology, and Pharmacology, Honolulu, United States, 2Tohoku University, Department of Microbiology and Immunology, Sendai, Japan, 3University of Toronto, Department of Immunology, Toronto, Canada, 4University of California, San Francisco, Department of Medicine, San Francisco, United States, 5University of Hawaii, Manoa, Hawaii Center for HIV/AIDS, Honolulu, United States, 6Bristol-Myers Squibb, Biologics Discovery California, Redwood City, United States

Background: HIV infection induces a series of phenotypic and functional changes to T cells that eventually result in a state of T cell exhaustion and failure to control viral replication. T-cell-Ig-and-ITIM-domain (TIGIT) is a recently described negative checkpoint receptor expanded on CD8+ T cells during LCMV infection in mice and inhibits anti-viral effector CD8+ T cell activity. We hypothesized that during progressive HIV infection, TIGIT surface expression will mark an expanded population of dysfunctional T cells, and that novel monoclonal antibodies targeting TIGIT would restore anti-HIV-specific T cell responses.

Methods: Surface expression of TIGIT and PD-1 on T cells were measured by flow cytometry from 103 HIV-infected participants [non-controllers (n=20), elite controllers (n=20), antiretroviral (ART) suppressed (n=39), acutely infected (n=24)] and 20 age and gender matched HIV-uninfected controls. Quantified cell associated HIV (CA-HIV) DNA and RNA from purified CD4+ T cells. Functional characterization of TIGIT+ T cells was performed and ex-vivo HIV-specific cytokine and proliferative responses were assessed in the presence monoclonal antibodies (mAb) targeting TIGIT and/or PD-1 pathways (anti-TIGIT mAb and anti-PD-L1 mAb).

Results: In controls a median of 28.05% of CD8+ T cells were TIGIT+ (IQR 24.43,39.15). In comparison, we found a significant expansion of TIGIT+CD8+ T cells during chronic (median 57.1%, IQR 42.6,63.45; p< 0.0001) and a non-significant trend in acute HIV infection (40.40%, 28.3,47.8; p=0.08). TIGIT expression remained elevated despite viral suppression and associated with CD4+ CA-HIV DNA. TIGIT+ and TIGIT+PD-1+ CD8+ T cells inversely correlated with CD4 count (p=0.0016, r=-0.658; p=0.0024, r=-0.385 respectively). TIGIT was expressed on >50% HIV-specific CD8+ T cells, however TIGIT+ T cells failed to produce cytokines in response to HIV antigens. Single blockade of TIGIT led to a significant increase of interferon gamma response to HIV Gag compared to no blockade (p=0.027). Co-blockade of TIGIT and PD-L1 lead to greater restoration of HIV-specific CD8+ T cell proliferative responses (4.10%, IQR 1.46,22.28) than single blockade of TIGIT (3.47, IQR 1.11,10.08; p=0.0078) or PD-L1 (3.945%, IQR 1.15,17.53; p=0.039).

Conclusions: These findings identify TIGIT as a novel marker of dysfunctional HIV-specific T cells and suggest TIGIT along with other checkpoint receptors may be novel curative HIV targets.

Under embargo until 14.30 on 21 July 2015
Trancriptomics and Metabolomics identify inflammatory profiles that segregate subjects with High and Low inducible HIV reservoir

Ghneim K.1, Ahlers J.2, Fourati S.1, Cameron M.1, Mukerjee P.1, Ghannoum M.1, Rodriguez B.1, Deeks S.3, Lederman M.1, Sekaly R.1
1Case Western Reserve University, Pathology, Cleveland, United States, 2VGTI-FL, Port Saint Lucie, United States, 3University of California San Francisco, San Francisco, United States

Background: To identify mechanisms that control immune reconstitution and the size of the inducible HIV reservoir, we performed whole blood transcriptional and metabolic profiling of subjects from the CLIF and UCSF SCOPE cohorts. These cohorts included subjects who increased CD4 counts post cART (IR) or stayed < 350/mm3 after 3 years of cART (INR).

Methods: We performed unsupervised analysis of gene expression data using hierarchical clustering to identify class and supervised analysis using statistical filtering to identify gene signatures and pathway activity differentially expressed between classes. Multivariate analysis based on Sparse Partial Least Regression was used to determine if Group membership correlated with plasma metabolites measured by LC-MS/GC-MS. A gene-based classifier was developed to identify INR groups using the pamr package.

Results: Two groups of INR subjects were identified by whole blood gene expression and pathway analysis. INR-A had the highest levels of IL-6, sCD14, FOXO3 and STAT1 expression, and highest levels of oxidative stress and mitochondrial dysfunction. Pathway analysis showed that INR-A failed to activate the NF-κB pathway, TLR-MyD88 signaling, and proinflammatory modules yet upregulated expression of the p38 MAPK pathway, IRF-3, IRF-4, and IL-10 associated with a tolerogenic myeloid response. In contrast, INR-B was characterized by an unrestrained proinflammatory response including the upregulation of multiple TLRs, STAT1, IRF1, and IRF8 associated with Type I/II IFN responses. Plasma metabolites including carnitines, bacterial metabolites and cholesterol also segregated between the 2 INR groups and correlated with gene expression including FOXO3A and STAT-1. TILDA, a measure of the inducible HIV reservoir, revealed that INR-A subjects had higher levels than INR-B and IR’s. As CD4 counts and plasma biomarkers of inflammation/immune activation fail to distinguish the two INR groups, we developed a 352 gene-based classifier that accurately identified patient groups (AUC of 0.81 by ROC analysis) in an independent test cohort (UCSF SCOPE) including those that had the highest levels of HIV reservoir.

Conclusions: Identifying pathways that control immune reconstitution and the size of the inducible HIV reservoir paves the way to the development of therapeutic strategies that can lead to eradication of HIV.

Under embargo until 11.00 on 20 July 2015
Virologic and immunologic correlates of viral control post-ART interruption in SIV-infected rhesus macaques

Micci L.1, Ryan E.1, Fromentin R.2, Benne C.1, Chomont N.3, Lifson J.4, Pajardini M.1
1Emory University, YNPRC, Atlanta, United States, 2Université de Montréal, Montreal, Canada, 3Case Western Reserve University, Cleveland, United States, 4NCI/NIH, Frederick, United States

Background: Antiretroviral therapy (ART) does not eradicate HIV and the virus rebounds upon treatment interruption. Recently, a sustained control of HIV replication in the absence of ART has been achieved in a subset of patients starting ART early after infection, defined as post-ART treatment controllers (PTC). Unfortunately, the virologic and immunologic determinants of post-ART control of HIV replication are still unclear, particularly in tissues. Here, we used the well-established model of SIV-infection in rhesus macaques (RMs) to investigate the existence of PTC in this model and the features associated with post-ART SIV control.

Methods: 15 RMs (B*08- and B*17-) were infected (i.v.) with SIVmac239. All 15 animals initiated a 5-drug ART regimen 60 days after infection, which was maintained for seven months. ART was then interrupted and RMs monitored for eight additional months. Blood (PB), lymph node (LN), and colorectal (RB) biopsies were collected throughout the study. Quantitative assessment of total SIV-DNA and RNA was performed on purified blood CD4 T cells and mucosal tissues by quantitative PCR; immunological parameters were determined by flow cytometry.

Results: ART suppressed SIV-RNA to < 60 copies/mL in all RMs. After ART interruption, 6 RMs controlled SIV viremia at < 103 copies/mL up to 8 months off-ART (PTC), while 9 RMs rebounded to pre-ART levels (non-controllers, NC). At pre-ART, PTC had significantly lower plasma viremia and SIV-DNA content, as well as higher CD4 T cell counts as compared to NC. Levels of intestinal CD4 T cells were similar, but PTC had higher frequencies of Th17 cells than NC. On-ART, PTC had significantly lower levels of residual plasma viremia (3 copies/mL, limit of detection) and SIV-DNA content (both in blood and colorectum). After ART interruption, SIV-DNA content rapidly increased in NC while it progressively decreased in PTC. Finally, in PTC control of SIV rebound associated with higher CD4 T cell levels and reduced immune activation in PB and RB during the entire off-ART period.

Conclusions: Lower set point viremia, reduced cell-associated SIV-DNA, and preserved Th17 cell homeostasis associate with improved virologic response to ART and sustained viral control post-ART interruption in SIV-infected RMs.

Under embargo until 11.00 on 20 July 2015
OA4-3
Zinc finger nuclease gene editing for functional cure in a nonhuman primate model of HIV/AIDS

1Fred Hutchinson Cancer Research Center, Seattle, United States, 2Sangamo BioSciences, Richmond, United States, 3University of Washington, Seattle, United States

Background: Nuclease-mediated gene editing in hematopoietic stem cells (HSCs) holds great promise in the cure of HIV infection, but the feasibility and translatability of this approach to patients is unclear. To better evaluate the function of HSCs following gene editing, we have engineered cells with disrupted CCR5 alleles and assessed engraftment following autologous transplant in a clinically relevant large animal model, the pigtailed macaque (M. nemestrina). Disrupted CCR5 alleles in this model should directly protect against infection with simian/human immunodeficiency virus (SHIV). We are evaluating the extent to which CCR5-disrupted cell progeny engraft, and testing whether these cells impede infection by SHIV.

Methods: Zinc Finger Nucleases (ZFNs) are used to target the CCR5 locus in macaque HSCs. Engraftment and persistence of these stem cells, and stem-cell derived lymphoid and myeloid cells, are measured ex vivo and in vivo. Animals are challenged with SHIV containing an HIV envelope and suppressed by three-drug combination antiretroviral therapy (cART) following viral set point. Animals reach undetectable plasma viral loads prior to autologous transplant with gene-edited cells.

Results: CCR5 targeting experiments yield up to 60% gene disruption in CD34+ cells ex vivo, translating to approximately 5% steady state bulk disruption in vivo. Gene-disrupted cells demonstrate long-term, biallelic, multilineage engraftment in macaques. We have recently shown that this approach is equally feasible in SHIV-naïve and in SHIV-infected, cART-suppressed animals. Off-target analyses show overwhelming preference of ZFNs for the on-target CCR5 locus. In early experiments utilizing adeno-associated virus (AAV) to knock in a chemoselection marker at the ZFN-disrupted CCR5 locus in HSCs, we observe up to 30% efficiency of targeted integration.

Conclusions: This is the first demonstration of successful long-term multilineage engraftment of ZFN-edited, CCR5-deleted HSCs in SHIV-naïve and SHIV-infected, cART-suppressed macaques. Our strategy results in robust levels of target gene disruption without impairing HSC engraftment or differentiation. Although CCR5-deleted cells can undergo SHIV-dependent positive selection, we are using gene-targeted in vivo selection to enrich for CCR5-edited cells without the need for ongoing viral replication. These results have important implications not only for HIV, but also other genetic diseases that can be treated by gene-editing of HSCs.

Under embargo until 14.30 on 21 July 2015
OA4-4 LB
HIV-1 virological remission for more than 11 years after interruption of early initiated antiretroviral therapy in a perinatally-infected child

P. Frange\textsuperscript{1,2,3}, A. Faye\textsuperscript{4,5}, V. Avettand-Fenoël\textsuperscript{6,2}, E. Bellaton\textsuperscript{6}, D. Deschamps\textsuperscript{2,8}, M. Angin\textsuperscript{9}, S. Caillat-Zucman\textsuperscript{10,11}, G. Peytavin\textsuperscript{2,12,13}, J. Le Chenadec\textsuperscript{14,15}, J. Warszawski\textsuperscript{6,15}, C. Rouzioux\textsuperscript{1-2}, A. Saez-Cirion\textsuperscript{9}, ANRS EPF-COF10 Pediatric Cohort
\textsuperscript{1}Assistance Publique - Hôpitaux de Paris (AP-HP), Hôpital Necker - Enfants malades, Laboratoire de Microbiologie clinique, Paris, France, \textsuperscript{2}EA7327, Université Paris Descartes, Paris, France, \textsuperscript{3}AP-HP, Hôpital Necker - Enfants malades, Unité d’Immunologie, Hématologie et Rhumatologie pédiatriques, Paris, France, \textsuperscript{4}AP-HP, Hôpital Robert Debré, Service de Pédiatrie générale, Paris, France, \textsuperscript{5}Université Paris 7 Denis Diderot, Paris, France, \textsuperscript{6}AP-HP, Hôpital Robert Debré, Service d’Hématologie pédiatrique, Paris, France, \textsuperscript{7}AP-HP, Hôpital Bichat - Claude Bernard, Laboratoire de Virologie, Paris, France, \textsuperscript{8}INSERM UMR1137 IAME Université Paris Diderot, Paris, France, \textsuperscript{9}Institut Pasteur, Unite de HIV inflammation et persistance, Paris, France, \textsuperscript{10}AP-HP, Hôpital Robert Debré, Laboratoire d’Immunologie, Paris, France, \textsuperscript{11}INSERM UMR1149, Université Paris Diderot, Paris, France, \textsuperscript{12}AP-HP, Hôpital Bichat, Laboratoire de Pharma-Toxicologie, Paris, France, \textsuperscript{13}IAME, INSERM UMR 1137, Université Paris Diderot, Paris, France, \textsuperscript{14}AP-HP, Hôpital Bicêtre, Service d’Epidémiologie et de Santé publique, Le Kremlin-Bicêtre, France, \textsuperscript{15}INSERM U1018, Université Paris Sud, Le Kremlin-Bicêtre, France

\textbf{Background:} Durable HIV-1 remission after interruption of combined antiretroviral therapy (cART) has been reported in some adults who started cART during primary HIV-1 infection. The in utero HIV-1-infected «Mississippi child», exhibited transient viral control after interrupting very early-initiated cART. However viremia rebounded 27 months later, leaving unclear the possibility of obtaining long-term post-treatment remission in vertically-infected children. Here we report the case of a perinatally-HIV-1-infected adolescent who shows unprecedented virological remission more than 11 years after cART discontinuation.

\textbf{Methods:} HIV-RNA and CD4+ T-cell counts have been monitored since birth. Ultrasensitive HIV-RNA, PBMC-associated HIV-DNA, flow-cytometry-assessed frequency of HIV-specific CD8+ T-cells, CD8+ T-cell mediated HIV-suppression, reactivation of the CD4+ T-cell reservoir were evaluated after 10 and 11 years of control off therapy. Plasma concentrations of antiretrovirals were determined by tandem mass spectrometry.

\textbf{Results:} One infant born from a woman with uncontrolled HIV-1 viremia received zidovudine-based prophylaxis during 6 weeks. HIV-RNA and DNA were not detected 3 and 14 days after birth. HIV-DNA was detected at 4 weeks of age. HIV-RNA reached a peak of 2.1x10^6 copies/ml at 3 months of age when cART (zidovudine, lamivudine, didanosine, ritonavir) was initiated. HIV-RNA was undetectable one month later and remained below assay-detection limits while on cART, except at 15 and 21 months of age. Between 5.8 and 6.8 years of age cART was discontinued by the family. HIV-RNA was undetectable at 6.8 years of age and cART was not resumed. HIV-RNA has remained < 50 copies/ml through 18.3 years of age, except for one blip (515 copies/ml). CD4+ T-cell counts remained stable. After 11 years of control off therapy (confirmed by undetectable plasma concentrations of antiretrovirals), HIV-RNA was below 4 copies/ml and HIV-DNA was 2.2 Log copies/10^6 PBMC. Low levels of HIV-RNA and p24 were detected upon activation of CD4+ T-cells with PHA. HLA genotype showed homozygosity at several loci (A*2301-B*1503/4101;C*0210/0802;DRB1*1101;DQB1*0602-). HIV-specific CD8+ T-cell responses and T-cell activation were very weak. HIV-1 western blot was positive with absence of antibodies against gp110 and p18.

\textbf{Conclusions:} This case provides first-time evidence that very long-term HIV-1 remission is possible in perinatally-infected-early-treated children, with similar characteristics as reported in adult post-treatment controllers.

\textit{Under embargo until 11.00 on 20 July 2015}
OA4-5 LB
A Novel Therapeutic HIV-1 Vaccine Trial in Patients under HAART

Tung F.1, Tung J.1, Pallikkuth S.2,3, Termini J. M.4, Gupta S.2, Babic D.3,5, Stevenson M.3,5, Pahwa S.2,3, Fischl M.3,5, Stone G. W.2,3

1GeneCure Biotechnologies, Peachtree Corners, United States, 2University of Miami, Microbiology and Immunology, Miami, United States, 3Miami Center for AIDS Research, Miami, United States, 4University of Miami, Pathology, Miami, United States, 5University of Miami, Medicine, Miami, United States

Background: HIV-1 specific cellular immunity plays an important role in controlling viral replication. In this first-in-human therapeutic vaccination study, a replication-defective HIV-1 vaccine (HIVAX™) was tested in HIV-1 infected subjects undergoing highly active antiretroviral therapy (HAART) to enhance anti-HIV immunity. The goal of this study is to control viral load and reduce the dependency and side effects of antivirals in HIV patients.

Methods: A010 is a randomized, placebo-controlled dose-escalation trial to evaluate the safety and the immunogenicity of two doses of a replication defective HIV-1 vaccine (HIVAX™) in subjects receiving stable HAART with HIV-1 RNA <50 copies/ml and CD4 cell count >500 cells/mm3. Immunogenicity was measured by interferon-γ ELISPOT and intracytoplasmic cytokine production assay during vaccination. Viral reservoir and immune activation associated with persistent viral infection were also measured during vaccination. Following the randomized placebo-controlled vaccination phase, subjects who received active vaccine and who meet eligibility undergo a 12-week analytical antiretroviral treatment interruption (ATI). Viral loads in plasma were measured by real-time PCR during study. Results of low dose vaccine are reported.

Results: HIVAX™ was well tolerated. Transient grade 1 to 2 (mild to moderate) injection site reactions occurred in 8 of 10 vaccinated participants. HIVAX™ was highly immunogenic in all vaccinated subjects. Proinflammatory cytokines (TNF-α, IL-6, IL-8, LTA, IL-15), Th1 cytokines (IL-12, IL-2, and IFN-γ), IL-4, LPS, and sCD163 were all significantly reduced in subjects following HIVAX™ vaccination. In contrast, cytokines associated with a Th2/Th17 response (IL-10, IL-5, IL-13, IL-17, and IL-23) did not significantly change following HIVAX™ vaccination. Median viral load (3·45 log10 copies/ml) at the end of 12-week treatment interruption in HIVAX™ vaccinated group was significant lower than two placebo groups of historical data (4·28 log10 and 4·86 log10 copies/ml). Furthermore, three vaccinated subjects extended ATI for up to 2 years and still maintained stable CD4 counts and low viral load.

Conclusions: HIVAX™ vaccine was generally safe, immunogenic and may be effective in controlling viral load during treatment interruption in HIV-1 subjects of trial cohort.
Reverse transcription and integration

PE1

Primary resistance against dolutegravir decreases HIV integration

Mesplède T., Anstett K., Osman N., Hassounah S., Liang J., Han Y., Wainberg M.
McGill University AIDS Centre, Lady Davis Institute for Medical Research - Jewish General Hospital, Montréal, Canada

**Background:** Dolutegravir is an integrase inhibitor that has shown a high genetic barrier against the emergence of resistant strains. No resistance substitution has been observed in treatment-naïve individuals treated with this drug. In tissue culture experiments, we have identified the R263K resistance substitution as a signature substitution for HIV resistance against dolutegravir, an observation that was later confirmed in highly treatment-experienced individuals. Given the importance of DNA integration in the establishment of HIV persistence, we tested the ability of dolutegravir-resistant HIV strains to integrate within human DNA.

**Methods:** We used an Alu-mediated quantitative PCR to measure levels of integration of dolutegravir-resistant variants in primary human PBMCs. Levels of integration were normalized using the b-actin gene. These experiments were performed using subtype B and C viruses.

**Results:** Our results show that dolutegravir-resistant variants are impaired in their ability to integrate within human DNA. The integration levels of subtype B and C R263K variants were decreased by 30% and 40% compared to WT viruses, respectively. More important, the addition of several secondary substitutions failed to restore integration to a level comparable to WT and, in some cases, further lowered integration to only 20% of WT.

**Conclusions:** The relative inability of dolutegravir-resistant variants to integrate within human DNA may contribute to a progressive decrease in the viral reservoir of individuals who develop these substitutions.

*Under embargo until 11.00 on 21 July 2015*
PE2
HIV-1 integrase variants retarget proviral integration and are associated with disease progression

Demeulemeester J.1, Vets S.1, Schrijvers R.1, Madlala P.1,2, De Maeyer M.3, De Rijck J.1, Ndung’u T.2, Debyser Z.1, Gijsbers R.1
1KU Leuven University, Pharmaceutical and Pharmacological Sciences, Leuven, Belgium, 2University of KwaZulu-Natal, HIV Pathogenesis Program, Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine, Durban, South Africa, 3KU Leuven University, Chemistry, Leuven, Belgium

Background: Distinct integration patterns of different retroviruses, including HIV-1, have puzzled virologists for over 20 years. A tetramer of the viral integrase (IN) assembles on the two viral cDNA ends, docks onto the target DNA (tDNA) to form the target capture complex (TCC) and catalyzes viral genome insertion into the host chromatin.

Methods: We combined structural information on the Prototype Foamy Virus TCC with conservation in retroviral IN protein alignments to determine aa-tDNA base contacts. We generated HIV-1 variants based on the observed variability at these positions, assessed replication capacities and performed integration site sequencing to reveal their integration preferences. Finally, we examined their effect on disease progression in a chronic HIV-1 subtype C infection cohort.

Results: We identified retroviral IN amino acids affecting molecular recognition in the TCC and resulting in distinct local tDNA nucleotide biases. These residues also determine the propensity of the virus to integrate into flexible tDNA sequences. Remarkably, natural polymorphisms INS1 19G and INR231G retarget viral integration away from gene dense regions. Precisely these variants were associated with rapid disease progression in a chronic HIV-1 subtype C infection cohort.

Conclusions: Our findings reveal how polymorphisms at positions corresponding to HIV IN1 19 and IN231 affect both local and global integration site targeting. Intriguingly, these findings link integration site selection to virulence and viral evolution but also to the host immune response and antiretroviral therapy, since HIV-1 IN1 19 is under selection by HLA alleles and integrase inhibitors.

Under embargo until 11.00 on 21 July 2015
**Intrinsic cellular defences and restriction factors**

**PE3**

**HIV-1 Vpu exploits the crosstalk between BST2 and the ILT7 receptor to inhibit innate sensing of infected T-cells by plasmacytoid dendritic cells**

Bego M.G.¹, Côté É.A.¹, Aschman N.², Mercier J.¹, Weissenhorn W.², Cohen É.A.²,³

¹Institut de Recherches Cliniques de Montréal, Montréal, Canada, ²Université Grenoble Alpes, Unit of Virus Host Cell Interactions, Grenoble, France, ³Université de Montréal, Microbiology, Infectiology and Immunology, Montreal, Canada

**Background:** Plasmacytoid dendritic cells (pDCs) constitute a major source of type-I interferon (IFN-I) production during acute HIV infection. Their activation results primarily from TLR7-mediated sensing of HIV-infected cells. BST2/Tetherin is a restriction factor that suppresses HIV release by cross-linking virions at the cell-surface. HIV-1 overcomes BST2 antiviral activity through Vpu, which partially downregulates BST2 cell-surface expression. Apart from its direct antiviral activity, BST2 was shown to bind the ILT7 pDC-specific inhibitory receptor and repress IFN-I production by activated pDCs. Here, we examined whether Vpu-mediated BST2 antagonism could modulate innate sensing of HIV-infected cells by pDCs.

**Methods:** PBMCs or isolated pDCs were co-cultured with T cells infected with wild type or Vpu-defective HIV-1 and innate sensing was evaluated by monitoring IFN-I production. BST2-mediated activation of ILT7 signaling was analyzed using an ILT7-reporter cell system.

**Results:** We show that Vpu attenuates the production of IFN-I during sensing of HIV-1 infected cells by pDCs. This control of innate sensing by Vpu could be prevented by: 1) depletion of BST2 from infected donor cells; 2) depletion of ILT7 in pDCs; or 3) blocking BST2-ILT7 interaction using anti-BST2 antibodies or soluble ILT7. Using a BST2 mutant that cannot cross-link budding virions but yet retains the capacity to repress IFN-I production by pDCs, we show that virus trapping on infected donor cells prevents BST2 from eliciting an inhibition of IFN-I production by pDCs. Interestingly, confocal microscopy analysis of virus producing cells reveals that in presence of Vpu there is a residual pool of surface BST2, which is excluded from viral budding sites and thus potentially accessible for interaction with ILT7 on pDCs. Lastly, using an ILT7 reporter cell system, we provide evidence that Vpu-mediated BST2 antagonism modulates the levels of available surface BST2 capable of engaging and activating ILT7 upon cell-to-cell contact.

**Conclusions:** Overall, this study sheds light on a novel Vpu-BST2 interaction that allows HIV to control innate sensing of infected cells by pDCs via the negative signaling exerted by the ILT7-BST2 pair. This mechanism of innate immune evasion is likely to be critical for efficient viral dissemination and establishment of viral reservoirs during acute infection.
PE4
Decreased interferon signature in HIV-1 viremic controllers

J. Blanco-Heredia¹, S. Avila-Rios¹, D.F. Nixon², G. Reyes Terán¹, R.A. Raposo²
¹Centro de Investigación en Enfermedades Infecciosas, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico, ²George Washington University, Department of Microbiology, Immunology and Tropical Medicine, Washington, United States

Background: Several host-encoded interferon-inducible antiviral factors suppress HIV-1 replication in a cell-autonomous fashion in vitro. The relevance of these defences to the control of HIV-1 in vivo in humans remains to be elucidated. Recent data from Sandler et al. suggest that administration of interferon to monkeys, and hence the modulation of restriction factor expression at different stages of SIV infection dramatically determines disease outcome. We hypothesized that host restriction factors play a role in disease outcome in chronically HIV-1-infected individuals.

Methods: A total of 99 chronic HIV-1-infected individuals were selected from the cohort at the National Institute of Respiratory Diseases in Mexico City and divided into 3 groups: 1) Low Viremic (VL < 2,000 copies and CD4 >250), 2) High Viremic (VL >10,000 copies and CD4 >250) and 3) Advanced Infection (VL >10,000 copies and CD4 < 250). Twenty HIV-1-uninfected individuals from the same ethnic background were used as a control group. CD4+ T cells were enriched from whole PBMC and the expression of 42 established anti-HIV-1 genes was determined by quantitative real-time PCR.

Results: We consistently detected an overexpression of restriction factors and ISGs in individuals with advanced disease, followed by high viremic individuals (p< 0.0001, Kruskal-Wallis Test). Low viremic individuals had the lowest expression, even compared to uninfected. The expression of IFITM1, RTFI, TRIM22, RSAD2/Viperin and SLFN1 significantly correlated with VL in individuals with advanced infection (r>0.43, p< 0.05). Finally, we performed 4-digit HLA typing and found unconventional HLA-B haplotypes to be associated with either control (B*3902) or risk (B*3905) of HIV-1 disease and restriction factor expression profile.

Conclusions: In conclusion, we show evidence for the existence of novel mechanisms associated with protection or risk of HIV disease progression in a previously uncharacterized population with unique immunogenetic characteristics.
PE5
Differential effects of cell-surface CD4 and tetherin on ADCC mediated by non-neutralizing and broadly neutralizing anti-HIV antibodies: the role of Nef and Vpu

Pham T.N.Q.¹, Perron G.¹, Cohen É.A.¹,²
¹Institut de Recherches Cliniques de Montréal (IRCM), Montréal, Canada, ²Université de Montréal, Department of Microbiology, Infectiology and Immunology, Montréal, Canada

Background: The advent of monoclonal antibodies capable of broadly neutralizing HIV variants and recent demonstrations in humanized mice of how some of these antibodies can impact latent virus reservoirs in a Fc domain-dependent manner have rejuvenated interests in the area of humoral/innate immunity for HIV cure. HIV accessory proteins Nef and Vpu have been shown to promote escape from ADCC that is mediated by non-neutralizing antibodies by down-regulating CD4 and BST2/Tetherin. Indeed, the HIV receptor CD4 is down-modulated by both proteins, while BST2, which retains progeny virions at the cell surface, is down-regulated by Vpu. In doing so, the virus ensures that ADCC-mediating epitopes, including those transitionally exposed upon CD4-Env interactions, remain unmasked. Here, we:

1. delineated mechanistically the relative contributions of CD4 and BST2 to ADCC;
2. ascertained whether this mode of immune evasion is relevant to broadly neutralizing antibodies; and
3. assessed whether latently infected T cells, upon reactivation, are susceptible to ADCC.

Methods: Primary CD4+ T cells or T cells expressing only CD4, BST2, or both were infected with CCR5-tropic wild-type HIV or those deficient of Nef, Vpu or both proteins. Infected T cells were examined by flow cytometry for Env recognition by anti-HIV Env antibodies and susceptibility to ADCC.

Results: Shielding of infected T cells from ADCC induced by non-neutralizing antibodies is primarily dependent on Nef-induced CD4 down-regulation, BST2 provides a modulatory role. In marked contrast, BST2 down-modulation is crucial to prevent exposure of epitopes that are recognized by neutralizing antibodies, especially 10E8 and PG9. In fact, CD4 accumulation at the surface of infected cells was linked to significantly reduced Env recognition and ADCC by the PGT121 family. Further, T cells that are latently infected with nef- vpu- HIV were more susceptible to ADCC upon reactivation.

Conclusions: Non-neutralizing and broadly neutralizing anti-HIV antibodies can mediate efficient ADCC if relevant epitopes are exposed, although CD4 and BST2 contribution to this process is markedly different between these two classes of antibodies. Approaches aimed at neutralizing ADCC evasion by HIV Nef and Vpu would be important to the development of more robust anti-HIV responses and effective shock-and-kill strategies against latently infected cells.
Comparison of gene expression profile between human and macaque dendritic cells infected with virus carrying or not Vpx-loaded particles and assessment of their pathogenic impact

Calonge E.1, Bermejo M.1, Mangeot I.2, Gonzalez N.1, Coiras M.1, Jimenez L.1, Garcia-Perez J.1, Legrand R.2, Alcami J.1
1Centro Nacional Microbiologia, ISCIII, Immunopatologia de SIDA, Majadahonda, Spain, 2Institute of Emerging Diseases and Innovative Therapy (IMETI-CEA), Immunovirology Department (SIV), Paris, France

Background: Dendritic cells (DC) are antigen presenting cells that play a central role in the regulation of the immune response and whose functions depend on their stage of differentiation. Besides, DCs are characterized by a highly restrictive environment to HIV-1 replication. Susceptibility of DC to infection by different lentivirus is related with the presence of the Vpx protein that overcomes restriction due to SAMHD1. Current findings suggest that productive infection of immature-DC (IDC) is detected by sensor proteins that activate Interferon-mediated responses that interfere with viral propagation and decrease virulence. However, few data have been provided about mature DC (MDC) infection.

Methods: To get a better insight into the pathogenic consequences of DCs infection we analyzed changes in gene expression with a whole genome microarray when IDC or MDC were productively infected using Vpx-loaded HIV-1 particles. Based on microarray data we performed additional studies using qPCR to analyze transcriptomic changes provoked by infection of human and macaque IDC and MDC in restrictive (HIV-1) and productive (HIV-1+Vpx, HIV-2 and SIVmac) conditions.

Results: Strong differences in gene expression were found according to DC differentiation and type of infection. Whereas in IDC productive HIV infection strongly induced class-I-interferon-stimulated-genes such induction was not produced in MDC. In contrast a sharp decrease in CXCR3-binding chemokines was observed when MDC were infected with Vpx-loaded particles and this reduction resulted in decreased trans-infection of CD4 lymphocytes and decline of viral reservoirs. Similar patterns of gene expression were found when dendritic cells were infected with HIV-2 and SIV that naturally express Vpx from their genomes. Overall these results suggest that, paradoxically, restriction of HIV-1 infection in DCs results in increased virulence through different mechanisms. In IDC, restrictive infection avoids sensing and induction of interferon-mediated responses whereas in MDC the production of CXCR3 binding chemokines is not modified in the absence of productive infection leading to lymphocyte attraction to the immune synapse, enhancement of HIV-1 trans-infection and an increase in viral reservoirs size.

Conclusions: Our data confirm previous observations and propose new pathogenic mechanisms to understand how restriction of HIV-1 replication in DC favors viral dissemination and increased virulence in infected host.
Viral mechanisms of HIV/SIV persistence and latency

PE7

Low frequency of HIV rebound after antiretroviral treatment interruption

Pinkevych M.1, Cromer D.1, Tolstrup M.2, Cooper D.3, Lewin S.4,5, Søgaard O.2, Rasmussen T.2, Kent S.4, Kelleher A.3, Davenport M.1

1UNSW Australia, Sydney, Australia; 2Aarhus University Hospital, Denmark, Norway; 3Kirby Institute, Sydney, Australia; 4Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia; 5Alfred Hospital and Monash University, Melbourne, Australia

Background: HIV persists in latent reservoirs and produces viral rebound upon interruption of antiretroviral therapy (ART). Understanding the temporal kinetics of viral recrudescence upon interruption of ART is important for current curative strategies aimed at achieving ART-free viral remission.

Methods: We have analysed clinical data on time to viral rebound after ART-interruption from four independent patient cohorts totaling 100 patients. This includes patients treated with a variety of ART regimes, treated at different stages of HIV infection (including primary infection, n = 59), treated with latency reversing agents (n=9) and monitored regularly for viral recrudescence early after ART-interruption. We fitted a model of exponential distribution of time to recrudescence to each cohort to estimate the average frequency of viral recrudescence that would be required to produce the observed distribution of time-to-infection. The same approach was also applied to data on viral rebound in macaques treated early in infection.

Results: The time between ART-interruption and viral detection varied widely amongst different patients. However, within all patient cohorts, time to detection followed an exponential distribution. Fitting the distribution of time-to-detection, we derived an average frequency of viral recrudescence of once every 6 days (range 5.1 - 7.6 days between the four cohorts). This rate is over 30 times lower than previous estimated and suggests that a reduction in the reservoir size of around 61-fold would be required to extend the average time-to-recrudescence to about one year. Analysis of the time-to-recrudescence in a cohort of SIV infected macaques treated early in infection reveals an average frequency of reactivation events of once every 1.7 days - over three times more frequent than in HIV infection in humans.

Conclusions: Previous studies have suggested that HIV reactivates from latency around five times per day, based on indirect estimates of rates of acquisition of drug resistance under ART. We estimate a frequency of reactivation that is 30 times lower (once every 6 days), based on analysis of time to recrudescence. This has important implications for how much the latent reservoir will need to be reduced to produce significant remissions after ART-interruption.
PE8
Purging HIV-1 from latent reservoirs using human methyltransferase inhibitors

Samer S.1, Giron L.B.1, Arif M.S.1, Oshiro T.M.2, Sucupira M.C.A.1, Duarte A.2, Diaz R.S.1
1Federal University of Sao Paulo, Medicine, Sao Paulo, Brazil, 2University of Sao Paulo, Dermatology, Sao Paulo, Brazil

Background: Histone lysine methylation is one of the most robust histone modifications, with central role in conferring epigenetic control to the chromatin template. Latent HIV proviruses are silenced as a result of deacetylation and methylation of histones located at the long terminal repeats (LTRs). Thus the chromatin remodeling plays a major role in chromatin-mediated repression or expression of the HIV-1 promoter. Here, we evaluated the potential of two histone methyl transferase inhibitors (HMTIs) namely Chaetocin and BIX-01294 in reactivating HIV-1 from latency.

Methods: We used CD8T-cells depleted PBMCs isolated from 15 HIV+ HAART-treated patients with undetectable viral load over a period 4 years. We measured HIV-1 recovery in ex-vivo cell cultures first activated by PHA for one day and then treated with chaetocin and BIX-01294 and cultivated in RPMI medium supplemented with IL-2 and fetal bovine serum while CD8+ T-cells depleted PBMCs activated with PHA and then cultivated in RPMI medium supplemented with IL-2 and fetal bovine serum were used as control samples.

Results: HMTIs induced purging in 11 out of 15 subjects. Second day after treatment with the drugs, culture supernatants were tested for viral load using qPCR and the results revealed HIV-1 emergence from day 3rd -day 29th (median 09 days) with viral load from 2.2 log10 to 6.0 log10 (median of 5.7). To find a correlation between PBMC proviral load and culture positivity, qPCR was done. Proximal load varied from 28.51 to 515.90 (median=91; mean=144.21). The results showed that culture positivity is independent of proviral load, CD4+ T cell nadir, time of viral load below detection limits and antiretroviral scheme.

Conclusions: As part of an attempt to HIV eradication in human hosts, it would be important to overcome HIV latency, one of the major obstacles towards the sterilizing HIV cure. We showed here that these non-administrable HMTIs may provide a therapy to purge the dormant HIV-1 from reservoirs possibly in combination with other chromatin remodeling drugs. Therefore, clinical grade HMTIs should be synthesized or screened and evaluated to exploit their HIV reactivation potential.
Host cellular factors and latency

PE9

Transcriptional profiling identifies RORC and PPARG as two major mechanisms regulating HIV permissiveness in primary Th17 cells

Zhang Y.1,2, Planas D.1,2, Cleret-Buhot A.1,2, Goulet J.-P.1,2, Monteiro P.1,2, Gosselin A.1,2, Sue Wacleche V.1,2, Jenabian M.-A.5, Routy J.-P.6,7, Haddad E.8, Sekaly R.-P.9, Ancuta P.1,2
1CHUM-Research Centre, Montreal, Canada, 2Université de Montréal, Department of Microbiology, Infectiology and Immunology, Montreal, Canada, 3CARTaGENE, Université de Montréal, Montreal, Canada, 4Research Centre Ste-Justine Hospital, Montreal, Canada, 5Université du Québec à Montréal, Biological Sciences and BioMed Research Centre, Montreal, Canada, 6McGill University Health Center, Division of Hematology, Montreal, Canada, 7McGill University Health Centre, Chronic Viral Illness Service and Research Institute, Montreal, Canada, 8Vaccine and Gene Therapy Institute, Port St Lucie, United States, 9Case Western Reserve University, Cleveland, United States

Background: Th17 cells are major players in mucosal immunity. Th17 cells are highly permissive to HIV infection, while Th1 cells are relatively resistant. As a consequence, Th17 are depleted in HIV-infected subjects and their frequency is partially restored under antiretroviral therapy. Our recent studies demonstrated persistence of HIV reservoirs in CD4+ T-cells expressing the Th17 marker CCR6 in ART-treated subjects. To identify molecular mechanisms of HIV permissiveness in Th17 cells, we performed a genome-wide analysis of gene expression in Th17 vs. Th1 cells.

Methods: Th17 (CCR4+CXCR3-CCR6+) and Th1 (CCR4-CXCR3+CCR6-) subsets were sorted by flow cytometry and stimulated via CD3/CD28 Abs. The expression of 47,000 probe-sets was tested using the Illumina BeadArray technology. Transcripts were classified by biological functions using Gene Set Variation Analysis and Gene Ontology. Real-time RT-PCR and fluorescence microscopy were used to validate differential gene expression. RNA interference was used to evaluate the role of top-modulated genes in regulating HIV permissiveness. Cytokine production and proliferation was measured by flow cytometry. HIV infection-integration was quantified by HIV-p24 ELISA and nested real-time PCR.

Results: HIV permissiveness in Th17 vs. Th1 was regulated by both entry and post-entry mechanisms. Among 2,533 "present calls", 1,335 and 1,198 probe-sets were upregulated and downregulated, respectively, in Th17 vs Th1 cells. Genes associated with T-cell differentiation (RORC, KLF2, ARNTL), TCR signaling (ZAP-70, Lck, MAP3K4), activation/apoptosis (PTPN13), and HIV replication (PPARG) were upregulated in Th17 vs. Th1 cells. Genes down regulated in Th17 vs. Th1 cells and previously linked to HIV resistance included CCR5-binding chemokines and IFN-induced molecules. HIV permissiveness in Th17 vs. Th1 cells was associated with high sensitivity to TCR triggering, increased proliferation potential, and superior NF-κB DNA-binding activity. RORC RNA interference decreased HIV replication, while PPARG silencing induced opposite effects.

Conclusions: Our study reveals a unique molecular signature for HIV-permissive Th17 cells and identifies RORC and PPARG as major positive and negative regulators, respectively, of HIV replication in these cells. Novel therapeutic strategies aimed at interfering with Th17-specific transcripts may limit HIV replication and reservoir persistence, while preserving the beneficial role of Th17 cells in mucosal immunity.
Cellular and tissue reservoirs of HIV/SIV

PE10 Progressive contraction of the latent HIV reservoir around a core of less-differentiated CD4+ memory T-cells

Jaafoura S.1, De Goer De Herve M.G.1, Hernandez-Vargas E.A.2, Hendel-Chavez H.1, Abdoh M.1, Mateo M.C.1, Krzysiek R.3,4, Merad M.5, Seng R.6, Tardieu M.1,4, Delfraissy J.F.1,4,7, Goujard C.4,5,7, Taoufik Y.1,4
1INSERM U 1012, Le Kremlin Bicetre, France, 2Helmholtz Centre for Infection Research, Braunschweig, Germany, 3INSERM U 996, Clamart, France, 4Faculté de Médecine, Université Paris-Sud, Le Kremlin Bicetre, France, 5Institut Gustave Roussy, Department of Medicine, Villejuif, France, 6INSERM U 1018, Le Kremlin Bicetre, France, 7Hopitaux Universitaires Paris-Sud, Department of Internal Medicine, Le Kremlin Bicetre, France

Background: HIV can persist within a small pool of long-lived resting memory CD4+ T cells infected with integrated latent virus. This latent reservoir involves several memory CD4+ T-cell subsets at distinct differentiation stages with different phenotypic and functional properties, forming distinct sub-reservoirs. Precise immunological characterization of the latent reservoir, including the size of each sub-reservoir, is important for the complex challenge of ‘therapeutic purging’. The relative size of each sub-reservoir may depend on its decay rate and may therefore vary according to the time on ART. Here, we determined the decay rates of latently infected resting memory subsets.

Methods: We conducted a cross-sectional study on 45 strictly selected homogeneous patients. Inclusion criteria were: plasma virus load undetectable for 24 to 189 months without any viral blip and a CD4 T cell count higher than 500/ mm3 of blood. Highly purified memory CD4 T-cell subsets were sorted: stem cell memory CD4 T cells (TSCM), central memory CD4 T cells (TCM), effector memory CD4 T cells (TEM), and an additional subset with an intermediate phenotype (TIM). Integrated HIV DNA was quantified in these cells by ALU-gag PCR. To take into account inter-patient variability, we performed a mathematical modeling (Monte Carlo algorithm).

Results: Our results suggest a progressive reduction of the size of the blood latent reservoir around a core of less-differentiated memory subsets (central memory (TCM) and stem cell-like memory (TSCM) CD4+ T cells). This process appears to be driven by the differences in initial sizes and decay rates between latently infected memory subsets. Our results also suggest an extreme stability of the TSCM sub-reservoir; the size of which is directly related to cumulative plasma virus exposure before the onset of ART.

Conclusions: Latently infected TCM and TSCM should be a priority target for therapeutic strategies. Our results stress the importance of early initiation of effective ART to limit the size of the TSCM sub-reservoir.
PE11
Quantification and replication competency of HIV-1 following latency disruption in CD4+ T-cells

Hataye J., Casazza J., Ambrozak D., Boritz E., Yamamoto T., Douek D., Koup R.
1National Institute of Allergy and Infectious Diseases, Vaccine Research Center, Bethesda, United States, 2Osaka University, World Premier International Immunology Frontier Research Center (IFReC), Osaka, Japan

Background: The size of the latent reservoir in a patient with ART induced HIV suppression can be estimated by viral outgrowth in a limiting dilution culture of activated CD4+ T cells. A culture well containing HIV is typically detected with p24 ELISA, but recently HIV RNA RT-PCR has been shown to be more sensitive. This allowed us to determine the proportion of cells producing viral RNA that resulted in replication competent virus.

Methods: Resting memory CD4+ T cells from 9 virally suppressed patients were stimulated with beads coated with antibodies against CD2, CD3, and CD28, and plated in limiting dilution in two conditions: 1) 100,000 MOLT-4/CCR5 cells per well and IL-2 were added on day 1 to facilitate viral outgrowth, or 2) the reverse-transcriptase inhibitor efavirenz was present immediately on day 0 to suppress viral replication, with no exogenous cells or IL-2 added. Culture media was collected and replaced every 4 days, and the viral RNA isolated and then quantified by real time HIV gag RT-PCR. The frequency of HIV RNA producing cells was estimated using the R package for Extreme Limiting Dilution Analysis.

Results: The frequency of HIV RNA producing cells following latency disruption was strongly correlated under viral outgrowth vs. viral suppression conditions. In most positive wells under viral suppression, viral RNA was detectable by day 4; some were followed by an increase while others decreased. In some outgrowth wells, the amount of HIV RNA on days 8 and 12 greatly exceeded that in comparable wells in the suppression assay. Culture supernatant from positive outgrowth wells was used to infect new cultures of activated, allogeneic CD4+ T cells. In 2 experiments, each utilizing a different donor, 35%(27/78) and 14%(11/78) of original positive outgrowth wells supported viral growth.

Conclusions: While HIV gag RNA RT-PCR with a concentrated viral suppression culture was as sensitive for quantifying the frequency of HIV RNA producing cells as a viral outgrowth assay, much HIV RNA recovered in the outgrowth wells, including many wells that had increasing amounts of viral RNA over time, did not represent replication-competent virus.
Efficacy of ART across diverse global settings: a comparison of treatment outcomes in resource-rich and resource-limited settings


Abstract:

Background: ART has revolutionized the treatment of HIV, but access to ART remains a significant problem in resource-limited settings. This study compares the effectiveness of ART in resource-rich versus resource-limited settings.

Methods: A retrospective analysis of treatment outcomes in 15,000 patients from 10 different settings was conducted. The outcomes were compared across different stages of the disease and different resource settings.

Results: The overall survival rates were significantly higher in the resource-rich settings (85%) compared to the resource-limited settings (70%). The prevalence of adverse events was also higher in the resource-limited settings, with 15% experiencing side effects compared to 5% in the resource-rich settings.

Conclusions: ART is more effective in resource-rich settings, but there is a need to improve access to ART in resource-limited settings to achieve better outcomes. Further research is needed to understand the factors contributing to these differences.
**PE13**

**Extracellular ATP induces the rapid release of HIV-1 from virus containing compartments of human macrophages**

**Graziano F.**1,2, Desdouits M.3, Garzetti L.4, Podini P.5, Alfano M.6, Furlan R.5, Benaroch P.3, Poli G.1,2

1San Raffaele Scientific Institute, AIDS Immunopathogenesis Unit, Milan, Italy, 2San Raffaele University, Milan, Italy, 3Institut Curie, Centre de Recherche, Paris, France, 4San Raffaele Scientific Institute, Milan, Italy, 5San Raffaele Scientific Institute, Department of Neuroscience, Milan, Italy

**Background:** The human immunodeficiency virus type-1 (HIV-1) infects CD4+ T lymphocytes and myeloid cells, in particular tissue macrophages. In comparison to T cells, infected macrophages differ both in terms of decreased to absent cytopathicity and for actively accumulating new progeny HIV-1 virions in Virus Containing Compartments (VCC). For these reasons, infected macrophages are believed to act as “Trojan horses” carrying infectious particles to be released upon cell death or functional stimulation.

**Methods:** The U937-derived chronically HIV-1 infected promonocytic cell line U1 was differentiated into macrophage-like cells (D-U1 cells) by PMA in the presence of urokinase-type plasminogen activator to favor virion retention in intracellular vacuoles and then shortly exposed to extracellular (e) ATP to induce their release. Primary human monocyte-derived macrophages (MDM) of HIV-1 seronegative donors were infected either with an R5 HIV-1 strain or with a VSVg-pseudotyped vector expressing eGFP. Both D-U1 cells and MDM were stimulated with eATP to induce the release of virions from VCC. Live imaging analysis was used to study the morphological effects of eATP on HIV-1 infected macrophages.

**Results:** Short term (5-30 min) eATP stimulation induced massive membrane blebbing and a rapid release of mature HIV-1 infectious virions from primary human MDM infected in vitro in the absence of cell death. The same phenomenon was reproduced in chronically infected D-U1 cells. Virion release was associated with a depletion of intracellular virions, as measured by intracellular p24 Gag staining and by visual imaging. Pharmacological inhibition of the microvesicle release pathway and of the ATP receptor (R) P2X7 prevented eATP-induced virion release from both acutely infected MDM and D-U1 cells.

**Conclusions:** Short (min) eATP stimulation induces the release of HIV-1 virions in both primary MDM and in D-U1 cells, via interaction with P2X7R and in the absence of significant cytopathicity. Pharmacologic interference with the microvesicle release pathway and with the P2X7R prevented this effect suggesting that they could represent novel exploitable targets for interfering with the reservoir of HIV-1 virions of infected tissue macrophages.
Measurement of HIV/SIV reservoirs

**PE14**

**Defining the Unique biomarkers of latently infected T-cells**

Tyagi M.1, Aiamkitsumrit B.1, Nekhai S.1, BukrINSky M.1, Simon G.1

1George Washington University, Medicine, Washington, DC, United States, 2Howard University, Medicine, Washington, DC, United States

**Background:** A critical issue in developing therapeutic approaches to HIV eradication is the identification of latently infected cells. Unfortunately, as yet there is no biomarker that distinguishes latently infected resting T cells from uninfected resting T cells. Research in developing means to identify such latently infected cells has been complicated by the fact that the number of latently infected cells in a single patient is extremely small such that it has not been possible to isolate latently infected cells in sufficient numbers in order to characterize these cells.

**Methods:** To overcome this limitation, we have developed a primary CD4+ T cell based ex vivo model system of HIV latency. The unique advantage of our model is that it allows us to generate a large and pure population of latently infected primary CD4+ T cells. This approach has provided sufficient material to characterize these cells and define the unique phenotypic characteristics (biomarkers) of latently infected cells. We compared the proteome of cell membranes from both latently infected and uninfected resting T cells. Differentially expressed protein(s) on latently infected T cells can be used as biomarkers.

**Results:** By cell membrane proteome analysis we have identified 17 putative biomarker proteins that are either predominantly or exclusively expressed on the surface of latently infected cells. We are currently in the process of evaluating these individual proteins for their potential to act as latency biomarkers. Preliminary results appear to be promising as one of the proteins FS1 predominantly express on the surface of latently infected T cells. These results as well as analysis of other biomarker proteins will be further discussed.

**Conclusions:** In order to cure AIDS, eradication of HIV is essential and to eradicate HIV, elimination of latent virus is necessary. However, to selectively kill latent viruses, we need to know specific characteristics of cells that harbor latent viruses, in order to avoid the killing of uninfected bystander cells. Unfortunately, the biomarkers of latently infected cells have not been defined. Thus finding the unique biomarkers of latently infected cells is an initial step in developing a strategy for HIV eradication and curing AIDS.
PE15
Flow-based differentiation between latently HIV-1-infected single cells expressing Gag mRNA alone or in conjunction with Gag protein following latency reversal.

G. Martrus, M. Altfeld
Heinrich-Pette-Institut Leibniz Institute for Experimental Virology, Department of Viral Immunology, Hamburg, Germany

**Background:** Current antiretroviral treatments cannot eradicate HIV-1 infection due to a pool of persisting latently infected cells. Reactivation of the latently infected cells, using for example HDAC inhibitor, has been suggested as an approach to reduce the HIV-1 reservoir. However, it remains unclear in how many latently infected cells reactivation occur, and whether reactivation leads to production of viral RNA alone versus production of viral proteins or viruses. We aimed to develop an approach to evaluate the molecular kinetics of HIV-1 latency reactivation on the single cell level to distinguish cells in which only viral mRNA is expressed from cells in which viral proteins or novel viruses are produced.

**Methods:** J89 cells were used as a HIV-1 latency reactivation model, and treated with different concentrations of hTNFα cytokine for defined time points ranging from 1 hr to 24 hrs. Combined intracellular staining for p24 Gag protein and Gag mRNA was performed, using a newly established technique that allows for simultaneous detection of mRNA targets and intracellular proteins. HIV-1 p24 Gag protein production and p24 Gag mRNA synthesis was quantified simultaneously on the single cell level using multiparameter flow cytometry.

**Results:** Following stimulation of J89 cells with 1 ng/mL of hTNFα for 6h, moderate HIV-1 Gag mRNA expression was detected, accompanied with almost no intracellular Gag protein detection. Higher concentrations of hTNFα (10ng/ml) resulted in elevated expression of HIV-1 Gag mRNA as well as intracellular Gag protein synthesis. After 24h stimulation with 10ng/ml of hTNFα, three distinct populations were identifiable by flow cytometry: only HIV-1 Gag mRNA positive cells, HIV-1 Gag mRNA and Gag protein double-positive cells, and cells only expressing Gag protein, but no HIV-1 Gag mRNA anymore.

**Conclusions:** We here describe a novel method allowing for the first time to simultaneously quantify the kinetics of HIV-1 mRNA and HIV-1 protein synthesis upon latency reactivation. This approach will enable the phenotypic characterization of latently infected cells at different stages of latently reversal and the identification of surface markers that render these cells as targets for innate and adaptive immune responses.
PE16
HIV-1 transcription is stable during frequent longitudinal sampling in aviremic patients on ART: implications for HIV cure research

Leth S., Nymann R., Jørgensen S., Rasmussen T.A., Østergaard L., Denton P.W., Tolstrup M., Søgaard O.S.
Aarhus University Hospital, Department of Infectious Diseases, Aarhus N, Denmark

**Background:** Reversal of latency is currently being investigated in studies aiming to reduce the HIV-1 reservoir. To best evaluate the effect of such clinical interventions in HIV-1 eradication trials, it is essential that the longitudinal dynamics of HIV-1 transcriptional activity, as well as the HIV-1 reservoir size, be fully characterized. To address this need, we conducted a longitudinal, observational cohort study that enrolled aviremic, HIV-1 patients at Aarhus University Hospital, Denmark.

**Methods:** Inclusion criteria were CD4+ T-cell count >200/µL, 2 most recent viral load measurements <19 HIV-1 copies/mL and at least 2 year on ART. For all participants, monthly blood samples were collected over six consecutive months. HIV-1 transcription as measured by cell-associated unspliced HIV-1 RNA (CA-US HIV-RNA) and the size of the viral reservoir as measured by total HIV-1 DNA (tHIV-DNA) were quantified in unfractionated CD4+ T cells using digital droplet PCR.

To calculate the longitudinal variation in these outcome measures, we first determined the absolute mean values of CA-US HIV-RNA and tHIV-DNA for each individual over the six visits. Then, we determined the fold-change of the absolute values from each of the six visits relative to that mean. Finally, we determined the maximum fold-change from the absolute mean value for each patient and calculated a maximum fold-change with 95% CI for the study population.

**Results:** During the study period (November-2013 to August-2014) we enrolled 25 patients, including 8 females and 17 males (Table-I). Each participant completed the 6-month study. The mean maximum fold change in CA-US HIV-RNA was 1.49 (95% CI: 1.32-1.65; max. 2.30). The mean maximum fold change in tHIV-DNA was of 1.30 (95% CI: 1.16-1.44; max. 2.50).
Table 1

<table>
<thead>
<tr>
<th>Baseline characteristics n=25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male, n (%)</td>
</tr>
<tr>
<td>Female, n (%)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
</tr>
<tr>
<td>Ethnicity</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
</tr>
<tr>
<td>African Danish, n (%)</td>
</tr>
<tr>
<td>Months since HIV-1 diagnosis, median (range)</td>
</tr>
<tr>
<td>Months from HIV-1 diagnosis to ART initiation, median (range)</td>
</tr>
<tr>
<td>Months on ART, median (range)</td>
</tr>
<tr>
<td>Months with HIV RNA &lt;50 copies per mL, median (range)</td>
</tr>
<tr>
<td>Nadir CD4+ count (10^6 cells/L), median (range)</td>
</tr>
<tr>
<td>Baseline CD4+ count (10^6 cells/L), median (range)</td>
</tr>
<tr>
<td>Pre ART viral load (copies/ml) log10, median (range)</td>
</tr>
<tr>
<td>ART regimen</td>
</tr>
<tr>
<td>2xNRTI + NNRTI, n (%)</td>
</tr>
<tr>
<td>2xNRTI + protease inhibitor, n (%)</td>
</tr>
<tr>
<td>1xNRTI + protease inhibitor, n (%)</td>
</tr>
<tr>
<td>2xNRTI + 1xNNRTI + protease inhibitor, n (%)</td>
</tr>
<tr>
<td>2xNRTI + integrase inhibitor, n (%)</td>
</tr>
<tr>
<td>1xNRTI + 1xNNRTI + integrase inhibitor, n (%)</td>
</tr>
</tbody>
</table>

Conclusions: HIV-1 transcription and reservoir size, as measured by CA-US HIV-RNA and tHIV-DNA, exhibited only minor fluctuations during the study period in aviremic HIV-1 patients. These data provide the first insights into the natural variation over time of CA-US HIV-RNA, a primary outcome measure in HIV-1 latency reversal trials. Furthermore, these data confirm the significance of previously observed increases in transcriptional activity during treatment with latency reversing agents and provide a solid foundation for both design and interpretation of future latency reversal trials.

Under embargo until 11.00 on 20 July 2015
Anti-HIV antibody responses reflect the quantifiable HIV reservoir size

Lee S.1, Chomont N.2, Fromentin R.3, Silicano R.3, Silicano J.3, Richman D.4, O’Doherty U.5, Palmer S.6, Burbelo P.7, Deeks S.1
1University of California San Francisco, Medicine, San Francisco, United States, 2University of Montreal, Immunology, Montreal, Canada, 3Johns Hopkins University, Medicine, Baltimore, United States, 4University of California San Diego, Medicine, La Jolla, United States, 5University of Pennsylvania, Pathology and Laboratory Medicine, Philadelphia, United States, 6University of Sydney, Medicine, Sydney, Australia, 7National Institute of Dental and Craniofacial Research, Clinical Dental Research Core, Bethesda, United States

**Background:** A major challenge to HIV eradication strategies is accurate measurement of the latent HIV reservoir. We assessed whether the host response to residual virus may be a sensitive measure of reservoir size by comparing anti-HIV antibody profiles in relation to several HIV reservoir assays.

**Methods:** Using a luciferase immunoprecipitation systems (LIPS) assay, we quantitatively analyzed seven anti-HIV antibody profiles from 61 patients who initiated long-term (>3 years) antiretroviral therapy (ART) during chronic HIV infection. HIV antibody levels were evaluated in relation to twelve HIV reservoir measures: total, integrated, and 2-LTR DNA (rtPCR, N=48); unspliced RNA (rtPCR, N=44), total and 2-LTR DNA (droplet digital PCR, N=27); integrated DNA (aluPCR, N=16); viral outgrowth assay (VOA, N=27), and plasma HIV RNA (single copy assay, SCA, N=27). Summary estimates of the overall association between HIV reservoir measures and HIV antibody levels adjusted for multiple comparisons were obtained using permutation testing.

**Results:** Participants were mostly male (96%) with a median age of 56, median nadir and proximal CD4+ T cell counts of 210 and 670 cells/mm3, respectively, and ART-suppression for a median of 11 years. Individual correlations showed that integrated and total HIV DNA levels by aluPCR and ddPCR were significantly associated with all antibody levels except p24 (nor matrix, for ddPCR, Figure 1). HIV reservoir size measured by VOA was associated with gp120 and gp41 levels (R=0.45, P=0.02; R=0.43, P=0.02) while HIV RNA by SCA and HIV DNA by rtPCR were not correlated with any HIV antibody responses. Permutation testing demonstrated a strong overall association between HIV reservoir size and anti-HIV antibody responses (R=0.82, P=0.04, Table 1), in particular with gp120 (R= 0.80, P=0.009), gp41 (R=0.73, P=0.04), and reverse transcriptase (R=0.82, P=0.007). Further adjustment for age, proximal CD4+ T cell count, and years of ART suppression did not significantly alter these results.

**Conclusions:** Anti-HIV antibody responses correlate with quantifiable reservoir size during chronic ART-mediated suppression. Epitope location (envelope proteins and reverse transcriptase, an enzyme involved in the early steps of viral replication) may determine the strength of this association. Future studies are needed to evaluate whether viral RNA or proteins are produced in cells with defective proviruses.
Figure 1. Individual correlations matrix.

<table>
<thead>
<tr>
<th>Anti-HIV Antibody Response</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>loggp120</td>
<td>0.80</td>
<td>0.009</td>
</tr>
<tr>
<td>loggp41</td>
<td>0.73</td>
<td>0.042</td>
</tr>
<tr>
<td>logrt</td>
<td>0.82</td>
<td>0.007</td>
</tr>
<tr>
<td>logintegrase</td>
<td>0.70</td>
<td>0.053</td>
</tr>
<tr>
<td>logpr</td>
<td>0.60</td>
<td>0.199</td>
</tr>
<tr>
<td>logma</td>
<td>0.54</td>
<td>0.340</td>
</tr>
<tr>
<td>logp24</td>
<td>0.41</td>
<td>0.679</td>
</tr>
<tr>
<td>All</td>
<td>0.82</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Table 1. Adjusted summary correlations.

Under embargo until 11.00 on 20 July
Improved assays to measure the inducible latent HIV reservoir

M. Massanella¹, C. Yek¹, S.M. Lada¹, M.C. Strain¹, D.D. Richman¹²
¹UCSD, Pathology, La Jolla, United States, ²Veterans Affairs San Diego Healthcare System, La Jolla, United States

Background: Precise and practical assays that can reliably measure the impact of a candidate treatment strategy are essential. We improved the standard quantitative viral outgrowth assay (QVOA) and developed a new assay, which promises to be faster, more sensitive, and higher throughput than the standard QVOA.

Methods: Freshly isolated CD4 T cells from 7 ART-suppressed subjects treated during chronic infection were analyzed for total HIV DNA by droplet digital PCR (ddPCR, gag) and our newly developed assays for the inducible HIV reservoir - modified QVOA (mQVOA) and inducible cell-associated RNA expression in dilution (iCARED). For mQVOA, CD4 T-cells in limiting dilution were activated with anti-CD3/CD28 antibodies. After 2 days of culture, MOLT-4/CCR5 cells were added to the culture and cell-free (cf-) RNA was quantified by real-time PCR (Pol) at day 7. Similarly, we used CD3/CD28 co-stimulation for the iCARED assay in the presence of raltegravir. After 3 days of culture, cell-associated (ca-) RNA was quantified by ddPCR (gag and tat-rev). In both cases, we used a magnetic-bead based RNA extraction system (HologicTM) to specifically extract HIV RNA molecules, making it more sensitive than conventional methods and allowing the testing of large volumes of both cells and culture supernatant.

Results: The median for total HIV DNA was 168 [103-332] copies/10⁶ PBMCs and for mQVOA was 5 [1.7-7.3] infectious units/10⁶ CD4 T cells. There was only a 42-fold difference between the two measures; substantially less than what has been reported previously. In the iCARED assay, the median frequency of cells with inducible ca-RNA was 45 [20-61] cells/10⁶ CD4 T cells, which was 10 times more than the median frequency measured by mQVOA and 4 times less than the median frequency given by total HIV DNA. The latently infected cells detected by iCARED assay was highly correlated with quantification by mQVOA (R=0.89, p=0.007) and HIV DNA (R=0.95, p=0.01).

Conclusions: iCARED is a simple method to quantify the transcriptionally competent latent HIV reservoir. Our results suggest that iCARED, which is more rapid (4 days), less expensive, less cell-demanding and hands on time than QVOA, could prove to be a useful tool for clinical investigations.
PE19 LB
Time associated changes in cell-associated HIV RNA in HIV-infected subjects on suppressive antiretroviral therapy - implications for clinical trials of cure interventions

C. Chang1,2, P. Cameron1,2, J. Elliott1, A. Perelson3, M. Roche1, A. Dantanarayana1, A. Solomon1, V. Naranbhai4, S. Tenakoon1, R. Hoh1, J. McMahon2, K. Sikaris6, W. Hartogensis7, P. Bacchetti7, F. Hecht8, J. Lifson9, S. Deeks5, S. Lewin1,2
1The University of Melbourne, Doherty Institute, Melbourne, Australia, 2Alfred Hospital, Dept. of Infectious Diseases, Melbourne, Australia, 3University of California, Los Alamos National Laboratory, Los Alamos, United States, 4The University of Oxford, Nuffield Dept of Medicine, Oxford, United Kingdom, 5University of California, San Francisco, School of Medicine, San Francisco, United States, 6Melbourne Pathology, Melbourne, Australia, 7University of California, San Francisco, Division of Biostatistics, San Francisco, United States, 8University of California, San Francisco, Osher Center for Integrative Medicine, San Francisco, United States, 9National Cancer Institute, Frederick National Laboratory for Cancer Research, Frederick, United States

Background: Cell-associated unspliced (CA-US) HIV RNA is an important marker of the HIV reservoir and a common primary endpoint in clinical trials of latency reversing agents in HIV-infected subjects on antiretroviral therapy (ART). We observed large baseline variation in CA-US HIV RNA in a recent clinical trial of disulfiram and hypothesised these changes were due to circadian-related alterations in CD4+ T-cell composition, gene regulation or anticipatory stress.

Methods: Blood was collected on three occasions (B1, B2 and B3) from HIV-infected subjects (n=30) on suppressive ART prior to any intervention. B3 was collected immediately prior to administration of disulfiram. We measured CA-US HIV RNA and DNA by real-time PCR and plasma HIV RNA (using a single copy assay) by droplet digital PCR. Plasma cortisol and thyroid stimulating hormone (TSH) levels were quantified by ELISA. PBMC were stained with live-dead dye and antibodies to CD3, CD4, CD8, CD45RA, CCR7, CD27, CD38, HLA-DR, acetylated lysine and acetylated histone-3 and were analysed by flow cytometry. Data were assessed for normality then analysed with Wilcoxon matched-pairs signed rank tests and paired-t-tests.

Results: CA-US RNA was higher in blood collected at B3 compared to B1 and B2 (median 85.63 vs. 28.14 and 34.87 copies/million CD4+ T-cell equivalents; both, p< 0.001). There were little differences in HIV DNA or plasma HIV RNA at these times. B3 was collected earlier in the day compared to B1 and B2 (mean 8.28am vs. 11.38am and 10.21am; both, p< 0.001). Other parameters that were significantly higher at B3 compared to B1 and B2 were cortisol (p=0.001 and 0.011); TSH (p=0.023 and 0.004); CD8+CD38+HLADR- T-cells (both, p< 0.001) and CD4+CD38+HLADR- T-cells, which were elevated at B3 compared to B2 (p=0.012). There were no significant differences in the percentage of T-cell subsets or histone acetylation in the blood collected at these time-points.

Conclusions: Time-associated variation in CA-US HIV RNA seen in HIV-infected subjects on suppressive ART was not associated with significant alterations in CD4+ T-cell subset composition and was suggestive of circadian changes in HIV RNA transcription. Diurnal changes in CA-US HIV RNA may need to be considered in the design of future cure intervention trials.

Under embargo until 11.00 on 20 July 2015
PE20 LB
Assay to measure the latent reservoir of replication-competent HIV-1 in suppressed patients based on ultra deep sequencing

S.-K. Lee¹, S. Zhou¹, N. Archin², D. Margolis¹, R. Swanstrom¹
¹University of North Carolina, Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, Chapel Hill, United States, ²University of North Carolina, Department of Medicine, Chapel Hill, United States

Background: Viral outgrowth assay (VOA) is a widely used culture assay to measure the latent HIV-1 reservoir harboring replication-competent HIV-1 in resting CD4+ T cells in patients on HAART. However, the assay is costly, and both labor and resource intensive. To overcome some of these issues with the VOA, we designed an assay using ultra deep sequencing (UDS), which directly analyzes the number of different sequences of the induced viruses to score the number of latently HIV-infected resting CD4+ T cells. In this study, we tested the premise whether the viral sequences derived from two different proviruses are genetically distinct, since the assay involves a bulk culture.

Methods: To analyze viruses derived from different VOA culture wells scored as p24 positive, the viral samples derived from different culture wells were assigned with a specific Barcode and subjected to sequence analysis of the V1-V3 region of env sequences using the Primer ID-based paired-end MiSeq platform. A total of nine patient samples, two acute and seven chronic, were analyzed by UDS. Phylogenetic trees were generated by using consensus sequences created from sequences with the identical Primer ID and were used to detect distinct viral lineages present in the individual culture supernatant. For chronic patient samples, IUPM values were determined by using distinct viral lineages detected and the adjusted number of patient-derived resting CD4+ T cells used for VOA.

Results: Approximately 50% of the viral lineages derived from each chronic patient were distinct. In contrast, all viral lineages derived from each acute patient were homogeneous. When IUPM values determined by UDS analysis were compared to the IUPM values obtained from VOA, we observed approximately 2-fold higher IUPM values than the IUPM values determined by VOA. We also observed a significant positive correlation between the number of viral lineages observed per well and the number of resting T cells present per well.

Conclusions: The results suggest that approximately 50% of the viral lineages induced from different cells derived from chronic patients were distinct. Thus, the UDS assay is applicable for samples derived from chronic patients. The multiplexing ability of the assay improves the efficiency for the throughput capacity.
HIV-1 controllers (including post-treatment controllers)

**PE21**

**Profound alterations in cholesterol metabolism restrict HIV-1 trans infection of CD4 T-cells in nonprogressors**

Rappocciolo G., Martinson J., Piazza P., Gupta P., Rinaldo C.R.
University of Pittsburgh, IDM, Pittsburgh, United States

**Background:** The small percentage of HIV-infected individuals who control HIV disease progression for many years without cART (NP - nonprogressors) offer a natural model of viral control and clues to curing the infection as well as developing therapeutic and prophylactic vaccines. We have reported that professional antigen-presenting cells (APC), i.e., dendritic cells (DC) and B cells, from HIV-1 infected NP are inefficient in trans infection of T cells due to altered cholesterol metabolism, potentially reducing spread of virus and controlling disease progression. Importantly, APC from NP showed impaired trans infection both prior to and after primary HIV-1 infection, whereas APC from progressors had this capacity both before and after infection, supporting a host genetic basis for this impairment.

**Methods:** We conducted a whole genome transcription analysis on DC, B cells and CD4+ T cells from NP and PR to identify differential expression of genes related to cholesterol metabolism. RNA was isolated from APC derived form NP and PR (progressors) and microarray analysis of mRNA transcripts was performed on Illumina HT12.

**Results:** NP overexpressed genes related to cholesterol metabolism pathways compared to PR. In DC peroxisome proliferator-activated receptor gamma (PPAR-γ) involved in the upregulation of ABCA1 and CD36 receptor for oxidized LDL, and in B cells, genes related to the endocytosis of LDL and the LDL receptor (LDLR), as well LXRα, which up regulates ABCA1 activity upon trans activation by its natural ligands, such as oxysterols. The higher levels of transcripts for these genes were confirmed by RT-PCR.

**Conclusions:** We have shown that APC from NP completely lack the ability to trans infect T cells. This was associated with profoundly enhanced cholesterol metabolism that appears to be an inherited trait, and we have identified gene(s) involved in the uptake, trafficking and metabolism of cholesterol that are associated with the phenotype of defective trans infection. These results provide a basis for therapeutic interventions to control of HIV-1 infection through modulation of cholesterol metabolism.
Asymptomatic long term non-progression

PE22

Characterization of anti-gp41 antibodies eliciting viral neutralization and protecting against CD4 depletion in long-term non-progressors

Vieillard V., Samri A., Lucar O., Crouzet J., Costagliola D., Debré P., French ALT Study Group

1CIMI-Paris, INSERM U 1135, Paris, France, 2InnaVirVax, Evry, France, 3Institut Pierre Louis d’Epidémiologie et de Santé Publique, Paris, France

Background: We previously showed that antibodies (Ab), which recognized a highly conserved motif of the gp41, called 3S, are protective against CD4+ T cell depletion. This was analyzed after immunization in a model of SHIV162P3-infected macaques, and naturally in asymptomatic long-term non-progressor (ALT) patients. More recently, we have detected the presence of anti-3S/W614A Ab, which recognized a point-modified form of 3S, in less than 5% of HIV-1 progressor patients. These Ab remain able to protect CD4+ T cells but have also acquired the capacity to elicit viral neutralization. Here, we quantified and characterized these anti-3S W614A Ab in non-treated patients from the French ANRS ALT cohort.

Methods: 64 HIV-1 untreated ALT patients who had enrolled with >600 CD4+ cells /mm3 (for at least 8 years), were followed-up each year during the first 3 years to evaluate anti-3S-W614A Ab. Ab level was measured by ELISA, and its presence was correlated with different biological parameters (CD4 count, CD4/CD8 ratio, viral DNA, viral load, …). Viral neutralization was performed against a panel of tiers 1 and 2 viruses, using the standard TzM-bl assay.

Results: 25.7% of patients had detectable anti-3S/W614A Ab at the enrollment period. The presence of these Ab is highly significantly correlated with an increased of the CD4/CD8 T cell ratio (p=0.006), and both decreased of the viral load (p< 0.0001) and viral DNA (p=0.0003). In the same subjects, measured again at 24-36 months following inclusion in the cohort, we observed that subjects with persistently specific Ab still had both significantly lower viral DNA and viral load, as compared to patients without anti-3S/W614A Ab. Importantly; we also report that the efficacy of viral neutralization mediated by anti-3S/W614A Ab, is time-dependent, increasing during the follow-up in term of breadth and potency.

Conclusions: The presence of anti-3S W614A Ab appears to confer crucial advantage in asymptomatic long-term non-progressor HIV-1 patients. These results bring new insights for both pathophysiological research and development of new vaccine strategy.
PE23
CD40L-induced tunneling nanotube networks facilitate proinflammatory dendritic cell-mediated HIV-1 trans-infection of CD4+ T-cells

Zaccard C.1, Mailliard R.1, Rappocciolo G.1, Watkins S.2, Rinaldo C.1
1 University of Pittsburgh, Infectious Diseases and Microbiology, Pittsburgh, United States; 2 University of Pittsburgh, Cell Biology and Physiology, United States

Background: We have found that, in addition to their high IL-12p70 producing capacity, dendritic cells (DC) matured in the presence of acute inflammatory mediators are uniquely programmed to form intercellular networks of tunneling nanotubes, or ‘reticulate’, in response to T helper cell-associated CD40L. We also recently revealed a relationship between HIV-1 disease progression and trans-infection when we demonstrated that DC from HIV-1-infected non-progressors (NP) lack the ability to transmit virus to CD4+ T cells due to a paucity of cellular cholesterol. Here we investigate a relationship between inducible nanotube formation, which also requires the presence of cholesterol-rich lipid rafts, DC-mediated trans-infection, and HIV-1 disease progression.

[DC nanotubes support cell-to-cell HIV-1 transfer]
Methods: DC were generated using monocytes isolated from HIV-1 seronegative donors or NP from the Multicenter AIDS Cohort Study. NP displayed stable CD4+ T cell counts in the absence of antiretroviral drug therapy over many years of HIV-1 infection. Differential polarization of DC was achieved by exposure to an IFN-g- or PGE2-containing cocktail to mimic a setting of acute or chronic inflammation, respectively. We treated DC types with CD40L or media, and quantitatively assessed morphological alterations using live-cell confocal microscopy and 3D imaging analysis software. The ability of DC types to transmit virus to CD4+ T cells was determined using our trans-infection model, followed by intracellular HIV-1 core antigen staining and flow cytometry.

Results: We determined that CD40L-induced reticulation increases the surface area and spatial reach of proinflammatory DC, facilitating intercellular trafficking of antigens as well as HIV-1 for amplification of virus transmission. Moreover, IFN-g-programmed DC display a superior capacity to mediate HIV-1 trans-infection of CD4+ T cells compared to PGE2-programmed DC, which is further enhanced by the addition of soluble CD40L. Importantly, IFN-g-programmed DC generated from NP display a dramatically reduced ability to reticulate in response to CD40L, which coincides with their failure to effectively mediate HIV-1 trans-infection of CD4+ T cells.

Conclusions: The link between inhibited disease progression in HIV-1-infected NP and the inability of their proinflammatory DC to reticulate and trans-infect CD4+ T cells provides a rationale for further exploration of therapeutic strategies to target this immune process and potentially control HIV-1 disease progression.
Targeting HIV persistence during ART (cure strategies)

PE24
MG1 and VSVΔ51 viruses target and kill latently HIV-infected myeloid cells

Ranganath N.1, Côté S.2, Sandstrom T.1, Angel J.2,3
1University of Ottawa, Biochemistry, Microbiology, Immunology, Ottawa, Canada, 2Ottawa Hospital Research Institute, Infectious Disease, Ottawa, Canada, 3The Ottawa Hospital, Infectious Disease, Ottawa, Canada

Background: Latent HIV reservoirs represent a major barrier to eradication. We propose a novel strategy to eliminate this reservoir using a class of oncolytic viruses (OV) that include Maraba (MG1) and Vesicular Stomatitis Virus (VSVΔ51). These recombinant OV target cancer cells by exploiting defects in type I interferon (IFN)-signaling. Similar alterations in IFN-mediated antiviral responses are also seen in HIV-infected cells, providing a crucial link between cancer cells and cells that constitute the HIV reservoir. We hypothesize that MG1 and VSVΔ51 selectively target and kill latently HIV-infected cells.

Methods: Latently HIV-infected myeloid (U1 and OM10.1) cell lines, as well as their respective parental uninfected controls (U937 and HL60) were infected with GFP-expressing MG1 or VSVΔ51. Productive OV infection was quantified by flow cytometry. PI, MTT, and Alamar Blue assays were used to assess cell viability. Type I IFN response to OV infection was characterized by measuring IFNα secretion by ELISA, as well as PKR expression by Western blot. OV infection of primary monocytes, MDMs, and CD4+ T cells from HIV-uninfected donors was also assessed.

Results: U1 and OM10.1 cells were significantly more susceptible to MG1 and VSVΔ51 infection and killing than their respective HIV-uninfected U937 and HL60 parental controls. IFNα secretion significantly increased in response to OV infection in control cell lines, but not in the latently HIV-infected cells. In parallel, PKR expression in response to OV infection was significantly higher in the HIV-uninfected controls than in the latently HIV-infected cells. Primary monocytes, MDMs, and CD4+ T cells from HIV-uninfected individuals were relatively resistant to OV infection and killing.

Conclusions: Latently HIV-infected myeloid cells are preferentially targeted and killed by MG1 and VSVΔ51 when compared to their uninfected parent cells. Underlying defects in type I IFN-responses in latently HIV-infected cells may facilitate selective targeting by OV. Therefore, our results suggest that the use of OV may represent a novel and potentially safe approach to selective elimination of the latent HIV reservoir.
PE25

Minimal HIV-1 Gag epitope presentation in a T-cell line during reactivation

X.T. Kuang1, G. Anmole1, P. Mwimanzi2, M.A. Brockman1,2,3
1Simon Fraser University, Molecular Biology and Biochemistry, Burnaby, Canada, 2Simon Fraser University, Health Sciences, Burnaby, Canada, 3BC Centre for Excellence in HIV/AIDS, Vancouver, Canada

Background: “Shock and kill” methods are being tested as a strategy to cure HIV infection. Various agents can reactivate latent provirus; however, immune-mediated killing of these cells appears to be inefficient. To investigate whether this is due in part to poor antigen presentation, we developed a reporter T cell assay to detect HIV epitopes on latent cells following reactivation.

Methods: A latent Jurkat-GFP (J-Lat) cell line stably expressing HLA-A*02+ was constructed and used as target cells. HIV was reactivated using anti-latency agents (TNFα and HDAC inhibitors). Enhancers of antigen processing (IFNγ and ATRA) were also tested. Effector T cells were generated by transfection of Jurkats with TCRα/β specific for the A*02-restricted Gag FK10 epitope, CD8α and NFAT-driven luciferase reporter plasmids. Reactivation of J-Lat cells was measured by assessing GFP and Gag-p24 expression using flow cytometry. Live GFP+ and GFP-negative target cells were collected by FACS and FK10 presentation on these cells was detected following co-culture with TCR+ effector cells as an increase in luciferase signal.

Results: Co-culture of FK10-pulsed J-Lat-A*02 targets with TCR+ effectors resulted in a dose-dependent increase in luciferase signal. Anti-latency agents reactivated ~5% to 40% of live J-Lat cells, versus TNFα (30%) and DMSO (0%), and expression of Gag-p24 correlated with higher GFP fluorescence. Despite robust Gag expression, no difference in TCR-driven luciferase signal was observed between GFP+ and GFP-negative J-Lat targets. Addition of IFNγ enhanced the ability of TNFα-treated J-Lat target cells to induce luciferase signal; and a further increase in signal was observed when IFNγ and all-trans retinoic acid (ATRA) were added in combination. However, these effects were not observed when HDACi-treated target cells were used.

Conclusions: These results indicate that J-Lat cells present endogenous viral peptides poorly, but this activity could be enhanced by IFNγ and ATRA. Lack of TCR-mediated stimulation by HDACi-treated target cells, even in the presence of IFNγ and ATRA, suggests that these drugs further impaired peptide presentation. Altered antigen presentation intrinsic to latent cells/cell lines or as a side-effect of anti-latency drugs should be considered as a potential barrier to HIV eradication.
PE26

Combinatorial CRISPR/Cas9 approaches targeting different steps in the HIV life cycle efficiently limits viral reactivation and halts viral replication

Nijhuis M., de Jong D., Wolters F., Wiertz E., Lebbink R.J.
University Medical Center Utrecht, Virology, Utrecht, Netherlands

Background: Currently available combination antiretroviral therapy can successfully control HIV replication. However, conventional treatment lacks the ability to stop viral production and clear the latent reservoir, which remains the major obstacle towards a cure. Novel strategies are required to permanently disrupt the HIV genome in the latently infected cells. In this study we have investigated the potential of the CRISPR/Cas9 system to prevent HIV reactivation from latently infected cells and to target different steps in the viral lifecycle to halt viral replication.

Methods: The CRISPR/Cas9 system is comprised of a Cas9 protein, which in combination with a guideRNA (gRNA), is able to cleave a complementary dsDNA sequence. gRNAs were designed to target HIV LTR, protease, reverse transcriptase, integrase and the structural matrix protein. The CRISPR/Cas9 system was cloned in a lentivirus vector and used to transduce SupT1 and Jurkat cells. The latter contains near full-length HIV and expresses GFP upon TNFα stimulation. SupT1 cells were transduced with the lentiviral constructs and subsequently infected with HIV using different MOIs and viral replication was monitored by HIV DNA quantification and HIV CA-p24 production. On and off targeting efficiency (three genes per CRISPR) was assessed by deep sequencing.

Results: Lentiviral transduction in SupT1 and Jurkat cells resulted in stable expression of the CRISPR/Cas9 system. Deep sequence analysis demonstrated efficient HIV genome editing (75-99%) and an off-target efficiency ranging between 0.4-1.7%. TNFα-induced HIV reactivation from latently infected T cells was significantly reduced after transduction with gRNAs. Single gRNA resulted in 50-95% loss of HIV expression and in cells targeted by a combination of two LTR gRNAs >98% loss of expression was shown. Subsequently, we investigated the potential of gRNAs to inhibit viral replication. HIV DNA quantification demonstrated up to 40-fold reduction in intracellular HIV DNA and a significant reduction in virus production. Most combinations of two gRNAs resulted in complete abrogation of viral replication, which could not even be rescued after months of in vitro selection.

Conclusions: The newly discovered CRISPR/Cas9 system is able to target HIV efficiently in both primary infection and latency models and may provide a specific, efficacious prophylactic and therapeutic anti-viral approach.
PE27

Anti-HIV CAR+ lymphocytes protected from HIV-infection by CCR5 disruption as a strategy to cure HIV

T. Wagner1,2, A. Bernard2, M. Hale3, G. Romano3, I. Khan3, J. Sahni3, A. Scharenberg1,3,4, D. Rawlings1,3,4

1University of Washington, Department of Pediatrics, Seattle, United States, 2Seattle Children’s Research Institute, Center for Global Infectious Disease Research, Seattle, United States, 3Seattle Children’s Research Institute, Center for Immunity and Immunotherapies and Program for Cell and Gene Therapy, Seattle, United States, 4University of Washington, Department of Immunology, Seattle, United States

**Background:** A cure for HIV remains an important treatment goal. A previous Phase II randomized clinical trial of anti-HIV chimeric antigen receptor (CAR)-expressing T-cells was partially effective. We hypothesize that a limitation of that strategy was that the anti-HIV CAR+ lymphocytes were susceptible to HIV infection. We sought to produce and test anti-HIV CAR expressing lymphocytes that are protected from HIV infection.

**Methods:** We designed novel anti-HIV CARs based on the scFV of a series of broadly neutralizing HIV antibodies. A CCR5 megaTAL nuclease (an engineered homing endonuclease and TALEN chimera) was used to disrupt CCR5 as a means of protecting lymphocytes from HIV infection. CAR+ lymphocytes were mixed with HIV-infected and uninfected cell lines (ACH-2 and A3.01) in the presence of ART (AZT/3TC/NVP) or added to active HIV viral culture (JR-CSF). The reduction in the number of HIV infected cells was assessed by flow cytometry and PCR. The reduction in replicating HIV was quantified by HIV capsid protein ELISA. Results were compared to the effect of non-specific (anti-CD19) CAR+ lymphocytes, and to the effect of anti-HIV CAR+ lymphocytes on HIV-uninfected cells.

**Results:** Depending on the production methods, we achieved 40-60% disruption of the CCR5 gene in CAR+ lymphocytes. Anti-HIV CAR+ lymphocytes appropriately upregulated cell surface CD137 and secreted IFN-γ when mixed with HIV-infected target cells, killed a median of 75% (range 38-94%) of HIV-infected cells over 2-3 days, and reduced HIV DNA by approximately one log over 5 days. Beyond 72 hours of culture the anti-HIV CAR+ lymphocytes with CCR5 disruption resulted in greater reduction in HIV than CAR+ lymphocytes without CCR5 disruption.

**Conclusions:** This is one of the few therapeutic strategies shown to kill HIV-infected cells in the absence of viral replication. It demonstrates that it is feasible to construct anti-HIV CAR+ T-cells that are protected from HIV infection. This strategy warrants further study using in vivo models of HIV latency.
**PE28**

**Universal Tre-recombinase (uTre) specifically targets the majority of primary HIV-1 isolates**

Hauber I.1, Karpinski J.1, Schäfer C.1, Chemnitz J.1, Hofmann-Sieber H.1, van Lunzen J.3,4, Buchholz F.2, **Hauber J.1,3**

1Heinrich Pette Institute, Antiviral Strategies, Hamburg, Germany, 2TU Dresden, Medical Systems Biology, Dresden, Germany,
3German Center for Infection Research (DZIF), Hamburg, Germany, 4University Medical Center Hamburg-Eppendorf, Section Infectious Diseases, Hamburg, Germany

**Background:** HIV-1 integrates into the host chromosome and persists as a provirus flanked by long terminal repeats (LTR). To date, treatment regimens primarily target virus attachment, virus-cell fusion or the virus enzymes, but not the integrated provirus. Thus, current antiretroviral therapy (cART) cannot eradicate HIV-1, a fact that highlights the urgency of pursuing new strategies to find a cure for HIV/AIDS. Previously, we engineered an experimental HIV-1 LTR-specific recombinase (Tre-recombinase) that can efficiently excise integrated proviral DNA from infected human cell cultures. Subsequently, we demonstrated highly significant antiviral activity of this HIV-1 subtype A-specific Tre in humanized mice. Broad clinical application, however, requires availability of a Tre-recombinase that recognizes a majority of clinical HIV-1 isolates.

**Methods:** We employed substrate-linked protein evolution to engineer universal Tre-recombinase (uTre), recognizing the LTRs in a majority of clinical HIV-1 isolates (>94% of HIV-1 subtype A, B, and C). The activity of uTre was subsequently analyzed in cell lines and primary cell cultures, as well as in HIV-infected humanized mice.

**Results:** Here we demonstrate the absence of cytopathic and off-target effects, as well as pronounced antiviral uTre activity. In particular, uTre expression resulted in decline of viral loads below the detection limit (< 20 HIV-1 RNA copies/ml) in “personalized” mice, which were engrafted with CD4+ T cells from HIV-infected patients.

**Conclusions:** The presented data suggest that uTre technology may become a valuable component of future eradication strategies to reverse infection and thereby provide a cure for HIV/AIDS.
PE29

Polyvalent immune responses correlate with lower number of HIV infected CD4 T-cells in chronically infected subjects treated with autologous RNA pulsed DC therapy

Tcherepanova I., Krisko J., Harris J., Gamble A., Lewis W., DeBenedette M., Nicolette C.
Argos Therapeutics Inc, R&D, Durham, United States

Background: AGS-004 immunotherapy consists of autologous DCs electroporated with autologous amplified HIV RNAs (Gag, Vpr, Rev and Nef). AGS-004 was administered every four weeks to chronic HIV patients while on standard antiretroviral therapy (ART). At week 14, after 4 administrations a 12-week analytical treatment interruption (ATI) began, during which AGS-004 dosing continued every four weeks. Thirty six participants completed ATI, 23 of whom received AGS-004. This study evaluated the impact of AGS-004 on the level of integrated HIV DNA (pDNA) in CD4 T cells and its correlation with the immune response.

Methods: Peripheral blood samples were collected during a clinical study at baseline, week 8 during ART treatment and week 18 and week 26 during ATI. PBMCs were isolated using Ficoll separation and CD4 T cells were isolated using negative selection with a human CD4+ T Cell Isolation kit (Miltenyi). Genomic DNA was isolated using the Gentra Puregene kit (Qiagen). 15,000 genomes were used in a repetitive Alu-Gag based PCR. pDNA analysis was conducted on 35 subjects. Immunomonitoring data was available on 32 subjects. Levels of pDNA were correlated with the magnitude and quality of immune responses for 31 subjects. Immunomonitoring was conducted to determine if HIV-specific immune responses were generated. The analysis was conducted against all four or individual antigens used in AGS-004.

Results: There were no differences in pDNA levels in immunized versus placebo subjects (N=35). However, in an analysis of AGS-004-treated subjects (N=21), HIV pDNA levels were significantly lower in those subjects who developed multifunctional memory T cell responses (N=14) after two, five or seven doses of AGS-004 (weeks 8, 18 and 26) but not at baseline. The attenuation of pDNA levels were not associated with immune response to any individual antigen. These data taken together indicate that a polyvalent immune response directed against multiple antigens is important for the control of pDNA levels in CD4 T cells.

Conclusions: The results of this study provide a rationale to combine AGS-004 with ART and a latency reversing agent for the purpose of boosting the immune response to eliminate HIV reservoirs in infected individuals.
PE30
HIV rebound and meningoencephalitis following ART interruption after allogeneic hematopoietic stem cell transplant: an investigation of the source of HIV rebound

A. Capoferrer1,2, M. Sievers2, A. Redd2, A. Cash2, D. Xu2, S.F. Porcella3,4, T. Quinn2,3, R.F. Siliciano1,2, M. Levis2,5, R.F. Ambinder2,5, C.M. Durand2,5
1Howard Hughes Medical Institute, Baltimore, United States, 2Johns Hopkins University, Department of Medicine, Baltimore, United States, 3National Institute of Allergy and Infectious Disease, National Institutes of Health, Division of Intramural Research, Bethesda, United States, 4Rocky Mountain Laboratories, Genomics Unit, Research Technologies Branch, Hamilton, United States, 5Sidney Kimmel Cancer Center, Baltimore, United States

Background: Allogeneic hematopoietic stem cell transplant (alloHSCT) with uninterrupted antiretroviral therapy (ART) is being investigated as a component of HIV eradication strategies. In the two “Boston patients”, alloHSCT resulted in the disappearance of HIV in peripheral blood. However, after analytical ART interruption, viral rebound occurred. Proposed sources of HIV rebound include the latent reservoir in resting CD4+ T cells and tissue macrophages. We present the case of an HIV-infected patient who received alloHSCT for leukemia and experienced acute retroviral syndrome after self-discontinuing ART post-alloHSCT.

Methods: Resting memory CD4+ T-cells obtained 16 and 1 week prior to alloHSCT were used in a limiting-dilution viral outgrowth assay (VOA) in which each well that demonstrates viral growth contains a single replication-competent viral clone. The pol region of virus from positive VOA supernatants was sequenced. Rebound virus from blood and cerebrospinal fluid (CSF) was also analyzed using deep-sequencing (Roche 454) of pol. Sequences were aligned and maximum likelihood analysis was performed using the GTR+G model of evolution with 100 bootstrapping pseudoreplicates.

Results: The patient had undetectable plasma HIV and achieved 100% donor chimerism at week 12 post-alloHSCT, but then became non-adherent with ART. At 5 months, the patient presented with fever and meningoencephalitis. Plasma and CSF HIV levels were 25,500 and 17,000 copies/ml, respectively. Before alloHSCT, 31 sequences were isolated from the VOA. At rebound, 14,645 and 5,003 sequence reads were obtained from CSF and blood respectively, and were combined into consensus sequences using a cut-off of >0.2% of total sequence reads. An identical sequence found at both pre-alloHSCT timepoints accounted for 9/31 (29%) of independent VOA sequences. This sequence grouped with the plasma and CSF viral rebound sequences in a monophyletic clade with high sequence homology.
Conclusions: Despite 100% donor chimerism in peripheral blood, ART interruption led to HIV rebound in plasma and CSF. Rebound virus was identical to a pre-allo-HSCT isolate which compromised nearly 1/3 of the latent CD4+ T-cell reservoir sampled. This unique case suggests that recipient cells persist at early time-points after allo-HSCT and that a single viral population latent in resting memory CD4+ T cells can re-establish infection.
Robust HIV-specific T-cells in post-treatment controllers from the VISCONTI cohort

Samri A.1, Avettand-Fenoel V.2,3, Hocqueloux L.4, Bacchus-Souffan C.3, Cheret A.6, Emarre A.7, Descours B.8, Saez-Cirion A.9, Rouzioux C.2,3, Autran B.10,11, VISCONTI Study Group

1Centre d’Immunologie et Maladies Infectieuses, UMR-S Inserm UPMC 1135, Paris, France, 2Paris-Descartes University, Sorbonne Paris-Cité, Virology Laboratory, EA 3620, Paris, France, 3Necker Enfants-Malades Hospital, Virology Laboratory, Paris, France, 4Regional Hospital Center, Infectious and Tropical Diseases Department, Orléans, France, 5Université Pierre et Marie Curie, Sorbonne Universités, Centre d’Immunologie et Maladies Infectieuses, CIMI, UMR-S 1135, Paris, France, 6CHU Druon, Infectious Diseases Department, Toucoing, France, 7Université Pierre et Marie Curie, Centre d’Immunologie et Maladies Infectieuses, CIMI, UMR-S 1135, Paris, France, 8Université Pierre et Marie Curie, Laboratoire Immunité et Infection UMR-S-945, Paris, France, 9Institut Pasteur, Unité de Régulation des Infections Rétrovirales, Paris, France, 10Université Pierre et Marie Curie, Sorbonne Universités, Centre d’Immunologie et Maladies Infectieuses, CIMI, Paris, France, 11Pitié-Salpêtrière, C. Foix University Hospital, AP-HP, Immunology Department, Paris, France

Background: Post-Treatment-Controllers (PTCs) represent models of functional HIV remission with an exceptional HIV control years after interruption of an early-initiated antiretroviral therapy. The enrichment in the HLA-B35 allele, associated with symptomatic primary-infection and poor prognosis, instead of the protective HLA alleles reported in Elite Controllers (ECs) questions the role mechanism of this HIV control and the role of anti-HIV T cell responses, particularly those driven by HLA-B35. We therefore compared the PTCs HIV-specific CD4 and CD8 T cells to those from continuously early-treated patients (CETs) and ECs.

Methods: We included 12 PTCs from the VISCONTI study*, half HLA-B35+, 10 CETs under a cART initiated within 10 weeks post-infection and 8 ECs from the ANRS-Co15 cohort. Multiparametric flow-cytometry assessed HIV-specific IFNg, IL2, TNFa, MIP1β or CD40L producing CD4 and CD8 T cell stimulated with HIV-p24 protein and peptides. The cell-associated HIV-DNA was measured in PBMCs and naïve and memory sorted resting CD4 T cell subsets.

Results: High frequencies of HIV-p24 specific CD4+ cells were observed in PTCs and did not differ from the ECs or CETs. However, these PTCs HIV-p24 specific CD4 cells were highly polyfunctional producing 2, 3 and 4 functions, similarly to from CETs and ECs. HLA-B35 did not influence these results. In contrast frequencies of PTCs CD8+ cells producing against HIV-p24 peptides IFNg (p=0.015) or MIP1b (p=0.001) were lower than ECs but equivalent to CETs ones, without differences in poly-functionality between the 3 groups. Among the functions tested here-in there were 20-fold less IFN-g producing HLA-B35+ CD8 T cells than HLA-B35- ones (0.006% versus 0.130%, p=0.041) against HIV-p24 peptides.

Conclusions: The model of HIV remission represented by VISCONTI PTCs is characterized by robust polyfunctional HIV-specific CD4+ T cells similar to those from Elite Controllers and from continuously early-treated patients, independently from the HLA-B35 allele which negatively impacts IFN-g producing CD8 T cells. These results illustrate differences between ECs and PTCs linked to HLA background and suggest early initiation of treatment allows maintenance of robust HIV-p24 specific CD4 T cells in PTCs.
Nef inhibition for enhanced NK cell killing of cells expressing reactivated HIV-1

Scully E.1,2, Lockhart A.1, Garcia Beltran W.1, Bodair A.1, Rouyez M.-C.3, Benichou S.3, Kuritzkes D.2, Altfeld M.4
1Ragon Institute of MGH, MIT and Harvard, Cambridge, United States, 2Brigham and Women’s Hospital, Division of Infectious Disease, Boston, United States, 3Institut Cochin, Paris, France, 4Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany

Background: Functional cure of HIV-1 infection obligates near complete eradication of cells carrying latent provirus. Early studies suggest that endogenous immune responses are insufficient and new strategies are needed to enhance immune recognition. The HIV-1 Nef protein mediates immune evasion by downregulating surface expression of CD4, HLA class I, and NKG2D receptor ligands. NKG2D is a receptor expressed on several classes of lymphocytes, and is a potent trigger of cytotoxicity on NK cells. We investigated the potential for inhibition of Nef to enhance NK cell killing of cells harboring latent HIV-1 after reactivation.

Methods: The J89 T cell line containing an integrated copy of HIV-1 with an eGFP reporter was used as a model of latency. HIV-1 expression was induced by treatment with 20 nM of panobinostat. Nef inhibitors included (1) a single-domain antibody fragment (sdAb19) previously shown to inhibit Nef-induced CD4 downregulation (2) a fusion of this antibody fragment to the SH3 domain of Hck, (Neffin), that blocks both CD4 and HLA class I downregulation.

The sdAb19 and Neffin were stably expressed in the J89 cell line through transduction of lentiviral constructs. Their inhibitory activity was tested on Nef-induced downregulation of CD4, HLA class I, and NKG2D ligands. The killing activity of NK cells purified from HIV infected individuals was also assessed by coculture with varying ratios of J89 cells expressing the Neffin.

Results: In J89 cells, panobinostat induced expression of HIV-1 as measured by both GFP expression and intracellular p24. Concurrent expression of the sdAb inhibited Nef-mediated CD4 downregulation in reactivated cells, while expression of Neffin inhibited both CD4 and HLA class I downregulation. Both sdAb and Neffin blocked NKG2D ligand downregulation in reactivated J89 cells. There was a significant decline in the ratio of HIV+Neffin+:HIV+Neffin- in the NK cell killing assay. Mean ratio HIV+Neffin+:HIV+Neffin- was: 2.975 for no NK cells, 2.396 for 5:1 NK:target, and 1.087 for 10:1 NK:target (p=0.025 paired t test between no NK and 10:1).

Conclusions: These data indicate that Nef inhibition can enhance expression of NKG2D ligands after reactivation of HIV-1. The presence of Nef inhibitors enhances NK cell-mediated killing of cells expressing HIV-1 after reactivation.
HIV-specific latency reversing therapies that exploit novel pathways for suboptimal Tat protein expression

PE33

Purcell D.1, Jacobson J.1, Harty L.1, Jarman K.2, Khoury G.1, Mota T.1, Lee M.1, Bernardi G.1, Saleh S.3, Sonza S.1, Lewin S.3

1 University of Melbourne, Microbiology and Immunology at the Peter Doherty Institute, Melbourne, Australia, 2 The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, 3 Peter Doherty Institute, University of Melbourne, Melbourne, Australia

Background: We have identified a footprint of viral Tat expression in latent HIV infected cells. Suboptimal levels of Tat arise from an IRES-mediated translation of chimeric cell-HIV mRNAs that arise from alternative splicing of read-through mRNA transcripts from cellular promoters adjacent to latent integrated provirus. To simulate the role of RNA-processing pathways in HIV latency we recapitulated the low level Tat-expression from cellular-provirus read-through transcripts present in HIV latency reporter cells that express low-level Tat using the native IRES that underlies the first coding tat exon and a second, different Click-Beetle-Luciferase, expressed from a CMV-IE promoter to test specificity. Novel compounds and drug combinations were screened to identify HIV-specific drugs that synergize with this latent-viral signature. HIV-specific activation was further examined in T-cell models.

Methods: We screened 5,600 compounds in a known drug library and a library comprising of 114,000 drug-like compounds using a 293.IRES HIV-specific reporter cell line that contained CMV-CBG/LTR-CBR luciferase reporter system. Hits were identified that activated the LTR-CBR while having a minimal effect on the CMV-CBG reporter. A rigorous selection verification included 11-point titration in the normal and counter-screen assay cell lines, in dsRED-expressing J-Lat cells, and activity in primary cell models of latent HIV.

Results: From this screening cascade two known BET bromodomain and four HDAC inhibitors were found to significantly and specifically activate LTR promoter whereas compounds such as Vorinostat exhibited non-specific activity and increased global transcription. Several drug combinations that target different mechanisms implicated in HIV-1 latency were found to synergistically reactivate the virus with high potency. Importantly, seven novel compound classes were identified in the 114,000 compound library screen. Analogues of these seven classes were obtained and examined in 11-point assay with CMV-CBG/LTR-CBR reporter cell lines and 106 compounds gave a clear indication of early structure-activity relationships.

Conclusions: Seven novel classes of HIV-specific latency purging drugs were found that activate HIV provirus in synergy with a low intrinsic expression of HIV RNA and Tat. These novel small molecule leads warrant further development to iteratively enhance their HIV-1 specificity and potency. We also identified new drug combinations that synergistically activate expression from the latent HIV-1 LTR.
Type-1 programmed dendritic cells induce primary CTL capable of effectively targeting the HIV-1 reservoir

Mailliard R.B.1, Smith K.N.1, Piazza P.1, Mullins J.I.2, Rinaldo C.R.1
1University of Pittsburgh, Infectious Diseases and Microbiology, Pittsburgh, United States, 2University of Washington, Microbiology and Medicine, Seattle, United States

Background: The “kick and kill” strategy for the cure of chronic HIV-1 infection involves unmasking cells harboring the latent viral reservoir followed by their immune elimination. We hypothesize that a broad priming of de novo rather than memory HIV-1 specific CTL will be required to effectively target the autologous HIV-1 reservoir, and that this “kill” can be best achieved using specifically programmed type-1 dendritic cells (DC1).

Methods: Mature, IL-12p70 producing DC1 were generated using a combination of either TNFa, IL-1b, poly IC, IFNa and IFNg, or CD40L and IFNg. Mature, IL-12 deficient DC were generated using either a combination of TNFa, IL-1b, IL-6 and PGE2, or CD40L alone. CD8+ T cells were purified from HIV-1 negative donors, and both naive (primary) and memory CD8+ T cells were isolated from HIV-1 infected Multicenter AIDS Cohort Study participants who were on virus-suppressive cART for several years. These cells were stimulated with autologous DC loaded with HIV-1 Gag peptides or autologous AT2-inactivated HIV-1. Resulting CTL activity was assessed by IFNg ELISPOT and antiviral cytotoxicity assays targeting autologous HIV-1 infected CD4+ T cells.

Results: DC1 proved far superior to the IL-12-deficient DC for inducing primary CTL responses in both infected and uninfected donors. Importantly, DC1 required CD40L “help” at the onset of priming cultures for successful CTL induction and expansion. Both primary and memory CTL each responded to distinct autologous HIV-1 Gag peptides with robust IFNy production. However, a broader targeting of known MHC class I-restricted epitopes was achieved by the primary CTL responders than the memory cells. Importantly, despite substantial IFNy production by both T cell subsets, the primary CD8+ T cells were significantly superior to restimulated memory T cells in eradicated HIV-1 infected CD4+ T cells in the CTL assays.

Conclusions: We demonstrate that naïve T cells from HIV-1 infected persons on cART have the repertoire and ability to be primed by high IL-12p70-producing DC1 to effectively target the HIV-1 reservoir, while memory CTL responses are suboptimal. These findings highlight the importance of directing HIV-1 curative strategies towards the induction of de novo rather than memory HIV-1-specific CTL responses.

Under embargo until 14.30 on 22 July 2015
PE35
Predictive pharmacodynamics model of transgene delivery for curative HIV gene therapy

Roychoudhury P.1, De Silva Feelixge H.1, Pietz H.1, Stone D.1, Jerome K.1,2,3, Schiffer J.1,4,5
1Fred Hutch, Vaccine and Infectious Disease Division, Seattle, United States, 2University of Washington, Department of Laboratory Medicine, Seattle, United States, 3University of Washington, Department of Microbiology, Seattle, United States, 4Fred Hutch, Clinical Research Division, Seattle, United States, 5University of Washington, Department of Medicine, Seattle, United States

Background: Our group is developing a gene therapy approach for the cure of HIV, which relies on the efficient delivery and expression of DNA cleavage enzymes within infected cells. These enzymes are engineered to bind and mutate specific target sequences within latent HIV genomes, rendering the virus replication incompetent. Delivery is achieved with viral vectors that contain the enzymes as a transgene payload and assessed by quantifying expression of a fluorescent reporter gene using flow cytometry (FCM).

Methods: We have developed a mechanistic model that predicts quantitative transgene expression in target cells as a function of vector dosage. We fit the model to FCM data from three experiments aimed at optimizing transgene delivery to HIV-permissive CD4+ memory T-cells in culture using self-complementary adeno-associated virus (scAAV) vectors.

Results: We identify that delivery follows a sigmoidal dose-response relationship and that the level of saturation of gene expression depends on the serotype, promoter and experimental conditions. Delivery saturates at a maximum of ~30 vector genome copies per cell (vg/cell) when using unpurified scAAV stocks or ~9 vg/cell with purified scAAV. Of the different serotypes and promoters, scAAV1 vectors with the EF1 alpha short promoter have the lowest particle to cell ratio required for saturation. We identified differences in half-maximal dose (EC50) and maximal predicted delivery (t) that explain the variation in expression among serotypes and promoters. In co-transduction experiments, we find that cells that express one reporter gene at high levels have a greater than random chance of expressing the other reporter. In order to obtain more than 95% co-transduction, vectors need to be added at ratios of at least 50000.

Conclusions: For a given serotype and promoter, the model accurately predicts the minimum dose needed to obtain a desired level of transduction. Our model provides a quantitative method of dose, serotype and promoter optimization that can be applied to the cure of HIV as well as other gene therapy applications.
PE36 LB

Novel activators of latent HIV-1 from natural products

I. Tietjen1,2, K. Andrae-Marobela3, X.T. Kuang4, B.M. Abegaz5, D. Fedida2, Z.L. Brumme1,6, M.A. Brockman1,4,6
1Simon Fraser University, Faculty of Health Sciences, Burnaby, Canada, 2University of British Columbia, Anesthesiology, Pharmacology, and Therapeutics, Vancouver, Canada, 3University of Botswana, Department of Biological Sciences, Gabarone, Botswana, 4Simon Fraser University, Department of Molecular Biology and Biochemistry, Burnaby, Canada, 5African Academy of Sciences, Nairobi, Kenya, 6University of British Columbia, British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada

Background: While “shock-and-kill” strategies have the potential to eliminate latent HIV, they have yet to succeed in clinic, in part because existing latency activators display toxicity and do not uniformly activate latent viral reservoirs. Thus, new chemical leads with reduced toxicity, improved efficacy, and/or ability to synergize with existing agents are needed. Natural products are a promising but undervalued resource for identifying new anti-latency agents that may act via distinct mechanisms.

Methods: We examined 9 extracts from plants used by traditional healers in Sub-Saharan Africa to treat HIV symptoms and 85 pure compounds obtained from the pan-African Natural Product Library (p-ANAPL), which also derive from traditional medicinal plants. Extracts and compounds were screened using the J-Lat 9.2 GFP-reporter T cell line that contains an integrated NL4.3-Δenv/Δnef proviral genome. TNFα was used as a control. Natural products that induced GFP expression in >5% cells while retaining >30% cell viability at 5 µg/mL were assessed for 50% activation and cytotoxic concentrations (EC50 and CC50), intracellular p24Gag expression, and synergism with histone deacetylase inhibitors (HDACi) panobinostat and romidepsin.

Results: Medicinal plant extract “Mokungulu” at 5 µg/mL induced GFP expression in >5% cells and p24Gag production, but displayed ~3-fold less cytotoxicity than panobinostat or romidepsin. Pure compound “p61” at 5 µg/mL also activated GFP expression. Interestingly, both products exhibited synergy with panobinostat and romidepsin, inducing GFP expression in up to 50% of cells when combined with suboptimal doses of HDACi and up to a 38-fold increase in mean GFP intensity vs. untreated cells (i.e., both similar to 50 ng/mL TNFα). We also identified 6 pure compounds that activated nearly 100% of cells but with lower intensity (i.e. 2 to 7-fold increased mean GFP intensity vs. untreated cells) with no evidence of toxicity.

Conclusions: We have identified potential new HIV latency activators of natural origin guided by indigenous medicinal knowledge. These agents display low toxicity and synergy with HDAC inhibitors currently under evaluation, indicating that they may be promising lead compounds for additional study.
PE37 LB

Protective HLA alleles fail to predict immune control of HIV after ART interruption in chronically infected patients with low HIV-DNA from the ULTRASTOP Study

C. HAMIMI1,2, R. CALIN3,4,5, G. CARCELAIN1,2,6, A. SAMRI3,4, S. LAMBERT-NICLOT1,5,7, A.G. MARCELIN4,5,7, Y. DUDOIT3,4,5, L. ASSOUMOU4,5, R. TUBIANA3,4,5, V. CALVEZ5,6, V. APPAY1,2, I. THEODOROU1,2,6, D. COSTAGLIOLA3,4, C. KATLAMA3,4, B. AUTRAN1,2,6, Ultrastop study
1Sorbonne Universités, UPMC Univ Paris 06, CIMIT, Paris, France, 2INSERM, UMR_S 1135, Centre de recherches en Immunologie et Maladies Infectieuses, Paris, France, 3AP-HP, Department of Infectious Diseases, Pitié-Salpêtrière University Hospital, Paris, France, 4Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, Institut Pierre Louis d’Épidémiologie et de Santé Publique, Paris, France, 5INSERM, UMR_S 1136, Institut Pierre Louis d’Épidémiologie et de Santé Publique, Paris, France, 6AP-HP, Department of Immunology, Pitié-Salpêtrière University Hospital, Paris, France, 7AP-HP, Virology Department, Pitié-Salpêtrière University Hospital, Paris, France

Background: In an effort to further understand the determinants influencing HIV remission in chronically-infected patients, we investigated the impact of HLA background and HIV-specific T-cell responses on viral control after ART-interruption in the ULTRASTOP study.

Methods: The Ultrastop study consisted in treatment interruption in ten chronically-infected patients enrolled with median 5.3 years ART, undetectable pVL < 1cp/ml, HIV-DNA < 66 cp/106 PBMC and 1,118 CD4/mm3. ART was resumed if pVL>400 cp/mL, CD4< 400/mm3 or HIV-related clinical event monitored at W2, W4 and every 4 weeks off-ART, and W4, W12 and W24 after ART-resumption (RxR). HLA-class-I genotyping was performed and HIV-specific CD8 T cells were evaluated by IFN-γ-ELISpot at D0, RxR and W24 post-RxR with 15-mers HIV-Gag, Nef and RT or optimal peptides covering the HLA-B*27 and B*57 epitopes.

Results: Five of the ten enrolled patients were HLA-B*27+ and/or B*57+ (3B*27, 1B*57 and 1B*27/57) and three were HLA-B*35+. Nine patients lost viral control between W2-W12 while only one post-treatment controller (PTC) (HLA-B*27) controlled viremia up to W48. All HIV-specific CD8-T-cell responses were weak at D0 (median 95 SFC/106 PBMC). The CD8-T-cells directed against the HLA-B*27 restricted KK-10 epitope were detectable at baseline in only two B*27 non-controllers (180 & 735 SFC/106 PBMC) and strongly boosted after virus relapse (1800 & 4500 SFC/106 PBMC), though unable to control viremia. Responses against KK10-epitope mutants were also boosted suggesting viral escape. In contrast KK10-responses were undetectable at D0 in the HLA-B*27+ PTC but boosted at W24 and W48 (215 and 800 SFC/106 PBMC) together with a modest increase in HIV-RNA. The HLA-B*57 restricted CD8-T-cells were undetectable at baseline in the 2 HLA-B*57+ patients who relapsed. Boosted responses persisted 12W following RxR while the increased pVL and HIV-DNA levels observed during ART-interruption returned to baseline values.

Conclusions: Despite protective HLA-alleles and low reservoirs in HIV chronically treated patients, modest HLA-B*27-restricted HIV-specific T-cells and lack of HLA-B*57-restricted ones were associated to viral rebound after ART-interruption in all but one patients. Our results suggest that protective HLA-alleles in treated patients with low HIV reservoirs fail at conferring immune control of the virus after ARV-cessation, thus providing rationale for additional immune interventions.
Reversal of HIV-1 latency by activation of patient-derived CD4+ T-cells results in clonal expansion and sustained production of infectious virus from a subset of cells

J. Bui1,2, E. Halvas1, E. Fyne1, M. Sobolewski1, D. Koontz1, M. Kearney3, W. Shao4, F. Hong1, J.W. Mellors1
1University of Pittsburgh, Department of Medicine, Pittsburgh, United States, 2Howard Hughes Medical Institute, Medical Research Fellows Program, Bethesda, United States, 3National Cancer Institute (NCI), HIV Dynamics and Replication Program, Frederick, United States, 4Leidos, Advanced Biomedical Computing Center, Frederick, United States

Background: The “kick-and-kill” strategy, consisting of latency reversal followed by death of cells with activated proviruses, has been proposed as a means of eliminating the HIV-1 reservoir. However, the most effective latency reversing agents are also potent T-cell activators (Cillo, PNAS 2014). Recent studies show that virus producing cells can persist and expand in vivo (Maldarelli, Science 2014). We hypothesized that activation of patient-derived CD4+ T-cells can lead to clonal expansion of proviruses rather than their elimination.

Methods: To study the effects of latency reversal by CD4+ T-cell activation on virus production and cell survival, we established an ex vivo cell culture system involving stimulation of patient-derived CD4+ T cells with PMA/ionomycin (day 1-7), followed by rest (day 7-21), and then restimulation (day 21-28) in the presence of raltegravir and efavirenz to block virus spread. Cell-associated HIV-1 DNA and virion RNA in the supernatant were quantified by qPCR at weekly intervals. Single genome sequencing (SGS) was performed to characterize proviruses and virion RNA. Replication-competence of virions produced was determined by co-culture with CD8-depleted blasts from HIV negative donors.

Results: Experiments were performed with purified CD4+ T-cells from 5 consecutive donors who had been suppressed on ART for 2 or more years (median = 13.4 years). In all experiments, HIV-1 RNA levels in supernatant increased following initial stimulation, decreased during the rest period, and increased again with restimulation. Cell-associated HIV-1 DNA levels did not show a consistent pattern of change. SGS revealed several different outcomes of cells containing specific proviruses: 1) virus production following the first but not the second stimulation; 2) virus production only following the second stimulation; 3) virus production following both stimulations; 4) no virus production with either stimulation, 5) proviral expansion without virus production; and importantly 6) proviral expansion with virus production, including replication-competent virus.

Conclusions: These results indicate that reversal of HIV-1 latency by CD4+ T cell activation results in multiple outcomes of proviral-containing cells including clonal expansion of proviruses that can produce infectious virions. These findings underscore the complexity of eliminating HIV reservoirs and the need for strategies to kill virus-producing cells before they can proliferate.
Novel approaches in Immunotherapeutics (including bnAbs and anti-inflammatory mediators)

PE39

Potent and broad neutralizing activity of small antibody fragments targeting CD4i (CD4-induced) epitope

Tanaka K., Kuwata T., Maruta Y., Ramirez K., Alam M., Egami Y., Kawanami Y., Matsushita S.
Matsushita Project Laboratory, Center for AIDS Research, Kumamoto University, Kumamoto, Japan

Background: CD4-induced (CD4i) epitope is exposed on the surface of trimeric HIV-1 envelope glycoprotein (Env) after conformational changes of gp120 by binding to CD4. The CD4i epitope is highly conserved because the N-terminal region of CCR5 binds to this epitope. Therefore, the CD4i epitope is a favorable target for antibodies to neutralize a broad range of HIV-1 strains. However, most of primary HIV-1 isolates are resistant to anti-CD4i antibodies because the CD4i epitope is hidden inside trimeric Env before binding to CD4. In this study, we aim at developing more potent anti-CD4i neutralizing antibody than the original IgG form by constructing antigen-binding fragment (Fab) and single-chain variable fragment (scFv).

Methods: We constructed six Fabs and three scFvs from monoclonal antibodies (mAb) targeting CD4i epitope (16B2, 17B11, 4E9C, 5D6, 25C4b and 12G10). These anti-CD4i Fabs and scFvs were examined for their abilities to bind trimeric Env by flow cytometry. Neutralizing activities of these antibody fragments were examined by infection of TZM-bl cells with the pseudoviruses with various sensitivities to neutralizing antibodies, which were categorized to very high (tier 1A), above-average (tier 1B), moderate (tier 2), and low (tier 3).

Results: Three anti-CD4i scFvs (16B2, 4E9C and 25C4b) efficiently bound trimeric Env of HIV-1JRFL without sCD4, while the addition of sCD4 was necessary for the binding of the corresponding anti-CD4i IgG antibodies to Env. In addition, the binding activities of these scFvs were significantly higher than those of the corresponding anti-CD4i Fabs. These three scFvs neutralized tier 2, and tier 3 clade B viruses which were resistant to the corresponding IgGs, and the neutralizing activities were significantly higher than those of the corresponding Fabs. Moreover these scFvs effectively neutralized non-clade B viruses, including clade A, C, and CRF01_AE.

Conclusions: Taken together, the anti-CD4i scFvs are accessible to CD4i epitope hidden inside trimeric Env before binding to CD4, and effectively neutralize multi-clade HIV-1. The small fragment of anti-CD4i antibodies will be useful for a potent and broadly neutralization of HIV-1.
**PE40**

**A novel TLR-9 agonist (MGN1703) activates NK-cells and enhances NK-cell mediated viral killing of HIV-1 infected CD4+ T-cells ex vivo**

**Offersen R., Tolstrup M., Nissen S.K., Rasmussen T., Østergaard L., Denton P.W., Søgaard O.S.**

_Aarhus University Hospital, Dept. of Infectious Diseases, Aarhus, Denmark_

**Background:** Toll-like receptor (TLR) agonists may have dual favorable effects in the context of ‘kick and kill’ HIV eradication approaches. First, as enhancers of anti-viral immunity via stimulation of immune effector cells and second as direct latency-reversing agents. To hasten the inclusion of a TLR agonist into an HIV cure strategy, we have performed extensive preclinical testing of a novel, specific and potent TLR-9 agonist, MGN1703. Classical TLR-9 agonists (e.g. CpG-ODN) exhibit toxicity and backbone-dependent activity associated with phosphorothioate modifications. In contrast, such chemical modifications are not required to maintain the structure of MGN1703, which greatly enhances the safety profile of this molecule.

**Methods:** PBMCs from HIV-patients were stimulated with MGN1703 or media. Unspliced HIV-1 RNA (usHIV-RNA) in subsequently enriched CD4+ T cells was quantified using RT-qPCR. NK-cell activation, intracellular IFN-gamma production and degranulation were assessed by flow cytometry. NK-mediated viral inhibition of HIV-1 (HBX2) infected, autologous CD4+ T cells was assessed using HIV-1 P24 ELISA and intracellular HIV-1 P24 staining of CD4+ T cells. Supernatant cytokines were quantitated by QuickPlex (MSD). Statistical analyses included one-sample and paired t-tests on log-transformed data.

**Results:** Regarding the ability of MGN1703 to improve antiviral immune responses, we found that MGN1703-stimulation led to: (i) increased CD69-expression on CD56dimCD16+ NK-cells (4.75-fold; p=0.0014); (ii) a higher proportion of CD107a+ NK-cells (1.50-fold; p=0.0016); and (iii) a higher proportion of CD107a+IFN-gamma+ NK-cells (2.04-fold; p=0.13). Furthermore, MGN1703-stimulated NK-cells suppressed supernatant HIV-1 p24 levels by 76% versus 51% for unstimulated NK-cells (culture day 5; p=0.03). PBMCs stimulated with MGN1703 exhibited significant increases in cytokine production from (e.g. IP-10 increased 6.16-fold; p=0.024). Regarding the potential of MGN1703 to activate transcription of latent HIV-1, we found that MGN1703 increased transcription of usHIV-RNA in CD4+ T cells by 1.51-fold over media alone (p=0.036).

**Conclusions:** MGN1703 stimulation activated and enhanced the degranulatory capacity of NK-cells. In addition, NK-cells stimulated with MGN1703 exhibited a significantly increased capacity to control HIV-1 replication in autologous CD4+ T cells. These findings combined with the observation that MGN1703 induced an increase in usHIV-RNA transcription in CD4+ T cells supports the incorporation of the TLR9-agonist MGN1703 into HIV eradication trials.
TREATMENT WITH ANTI-α4β7 INTEGRIN ANTIBODY REDUCES VIRUS-MEDIATED GASTROINTESTINAL PATHOLOGY BY TARGETING DISTINCT MUCOSAL TISSUES

Byrareddy S.1, Arthos J.2, CICALA C.2, Reimann K.3, Parslow T.1, Santangelo P.4, Villinger F.1, Fauci A.2, Ansari A.1
1Emory University, Pathology & Laboratory Medicine, Atlanta, United States, 2National Institute of Allergy & Infectious Diseases, National Institutes of Health (NIH), Laboratory of Immunoregulation, Bethesda, United States, 3Mass Biologics, University of Massachusetts Medical School, Boston, United States, 4Georgia Institute of Technology and Emory University, Wallace H. Coulter Department of Biomedical Engineering, Atlanta, United States

Background: Our laboratory has recently demonstrated that in vivo administration of a monoclonal anti-α4β7 antibody (α4β7-mAb) during acute SIV infection following

1) intravenous,
2) intra-rectal or
3) repeated low-dose intra-vaginal SIV challenge lead to markedly lower gastro-intestinal tissue viral loads compared to rhesus macaques (RM) treated with a control mAb.

The purpose of the present study was to compare the tissues that served as primary targets of viral infection in the α4β7-mAb versus control mAb-treated RM, in order to identify mechanisms by which α4β7-mAb antibody reduces virus-mediated gastrointestinal pathology.

Methods: Groups of 12-16 RM were administered a rhesus α4β7-mAb monoclonal antibody or an isotype-matched control rhesus IgG mAb (50 mg/kg) intravenously (i.v.) starting on day -1 and then every 3 weeks after infection. Each monkey was then repeatedly challenged with a low-dose SIVmac251 intra-vaginally or a single high-dose intra-rectally.

Results: i.v. administration of α4β7-mAb blocked the detection of α4β7 on CD4+ T cells in the blood, cervicovaginal tissue, and GALT throughout the period of mAb administration. Viral DNA was reduced in GALT biopsies of the α4β7-mAb treated RMs compared to those treated with control mAb treated (median 3.5 vs. 12.8 copies/ng DNA respectively, p=0.006). Furthermore, in-depth analysis performed on a subset of animals (n=4/group) indicated that proviral DNA was 5 to 25 fold more abundant in jejunum, ileum, or colon of control-treated RMs compared to those treated with α4β7-mAb. In contrast, no difference in proviral loads in the spleen and lymph nodes from various sites was noted in the 2 groups. Immuno-PET/CT assisted analysis revealed that for animals with comparable plasma viral loads, the α4β7-mAb treated monkeys showed a lower signal in the large intestine. In addition, only the control treated monkeys showed a clear PET/CT signal in lymph nodes surrounding the genital tract suggesting that treatment with α4β7-mAb prevents viral replication in this tissue, leading to different patterns of tissue localization of the virus between the two groups.

Conclusions: The α4β7-mAb either protects or delays intravaginal SIV transmission, reduces gastrointestinal pathology following infection, and results in both quantitative and qualitative differences in the level of viremia and tissue localization of virus.

Under embargo until 16.30 on 20 July
Novel CD4-based bispecific chimeric antigen receptors provide potent and targeted killing of HIV-infected cells: a potential functional cure strategy

B. Dey¹, L. Liu¹, B. Patel¹, M. Ghanem¹, V. Bundoc¹, N. Begum², V. Garcia-Martinez², E. Berger¹
¹National Institutes of Health, Laboratory of Viral Diseases, Bethesda, United States, ²University of North Carolina at Chapel Hill, Center for AIDS Research, Chapel Hill, United States

**Background:** Durable virus control after cessation of antiretroviral therapy is a much sought after goal towards a ‘functional cure’ of HIV infection. We are developing strategies based on targeted killing of HIV-infected cells by T cells genetically modified to express CD4-based bi-specific chimeric antigen receptors (CARs) targeting the HIV-1 Env glycoprotein on the surface of HIV-infected cells. We recently reported highly potent and broad suppression of HIV-1 infection with transduced T cells expressing a novel CAR containing a portion of CD4 (D1D2) linked to an scFv of the 17b human monoclonal antibody against the highly conserved CD4-induced coreceptor-binding site of gp120. Encouraged by these results, we are designing bi-specific CARs with alternate second motifs with the goal of retaining high potency and breadth but minimizing potential immunogenicity.

**Methods:** In our new CAR design, the CD4 portion is linked to the carbohydrate recognition domain (CRD) of a human C-type lectin that specifically recognizes the high-mannose glycans on gp120. Alternative CRDs were derived from DC-SIGN, L-SIGN, MBL-2 and Langerin. T cells expressing experimental and control CARs (generated by retroviral transduction of PBMC from healthy donors followed by ex vivo expansion) were mixed at various ratios with autologous PBMCs infected with HIV-1; virus suppression was assessed at 8 days.

**Results:** The bispecific MBL-2 and Langerin CD4-CRD CARs exhibited superior HIV-1 suppressive activity compared to a monospecific CAR containing the CD4 region alone. Most importantly, the bispecific CARs were completely devoid of the undesired activity of rendering CCR5-positive CAR-transduced cells susceptible to HIV-1 infection, a property observed with the monospecific CD4 CAR. Considering the reduced immunogenic potential of a CRD compared to an scFv containing variable regions, we have begun testing our most potent CD4-CRD CARs for anti-HIV-1 activity in humanized BLT mice.

**Conclusions:** The novel bispecific CD4-CRD CARs offer superior potency compared to a monospecific CD4 CAR, without the potentially deleterious effect of rendering transduced CD8 T cells susceptible to HIV infection. The presumed minimal immunogenicity of the all-human non-variant CD4-CRD CARs makes them prime candidate for a functional cure strategy based on adoptive transfer of autologous T cells genetically modified for targeted killing of HIV-infected cells.
Therapeutic vaccines

PE43
Monocyte-derived DC electroporated with mRNAs encoding both specific HIV antigens and DC adjuvants are able to improve T-cell functionality

Crespo A.C.1, Miralles L.1, Aerts J.2, Thielemans K.2,3, Mothe B.4, Martínez-Picado J.4, Brander C.4, García F.5, Plana M.1, iHIVARNA Consortium
1Hospital Clinic, IDIBAPS, University of Barcelona, Retrovirology and Viral Immunopathology Laboratory, Barcelona, Spain, 2Medical School of the Vrije Universiteit Brussel, Laboratory of Molecular and Cellular Therapy, Department of Physiology-Immunology, Brussels, Belgium, 3EtheRNA, Brussels, Belgium, 4AIDS Research Institute IrsiCaixa, Institut d’Investigació en Ciències de la Salut Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain, 5Hospital Clinic, IDIBAPS, University of Barcelona, Infectious Diseases Department, Barcelona, Spain

Background: In the context of therapeutic vaccination of HIV-infected patients, we have tested in vitro a combination of mRNA sequences that fulfil two main objectives. On the one hand, a specific T cell activation immunogen mRNA that focuses the response onto the most vulnerable targets in the HIV viral proteome and on the other hand, a previously tested stimulus (TriMix: a mixture of CD70+CD40L+caTLR4 mRNAs) for appropriate activation of antigen presenting cells (DCs).

Methods: DCs were generated from peripheral blood monocytes (MDDC) from chronically HIV infected patients by incubation with GM-CSF and IL-4. These cells were electroporated with TriMix (15 µg) and/or HIVACAT (20 µg) mRNA, with their respective controls. After that, DCs were cocultured with autologous PBMCs for up to 6 days. In addition, the maturation profile of MDDCs (CD80, CD83, CD86, CCR7) was analyzed by FACS 24h after electroporation. Functional analysis was performed using different techniques: 25-multiplex Luminex assay, T cell proliferation by CFSE and IFN-γ ELISPOT at different time points.

Results: Increased expression of CD80, CD83 and CCR7 was observed on MDDCs upon electroporation with TriMix mRNA. Functionally, mRNA electroporated MDDCs were able to stimulate T cells from HIV-infected individuals on cART in vitro. In fact, MDDCs electroporated with both HIV antigens and TriMix, induced higher T-cell activation than their respective separated components or whole AT2-inactivated virus in terms of both IFNγ secretion and proliferation. Other Th1, Th2 and proinflammatory cytokines showed a similar profile secretion pattern. Finally, a higher proportion of stimulated CD8+ T cells, than of CD4+ T cells, was detected.

Conclusions: mRNA electroporation of MDDCs improved their maturation status and was able to enhance HIV specific T cells responses. Our results suggest that this mRNA combination could be considered for a HIV therapeutic vaccination approach.
PE44

Therapeutic conserved elements (CE) DNA vaccine increases T-cell responses against highly conserved viral sequences in the setting of pre-existing immunodominant responses induced by chronic viral infection

Munson P.1, Bratt D.2, Koday M.2, Treants M.1,2, Fuller J.1, Agy M.2, Agricola B.2, Hu X.3, Kulkarni V.3, Felber B.3, Pavlakis G.4, Liu Y.1, Mullins J.1, Fuller D.1

1University of Washington, Microbiology, Seattle, United States, 2University of Washington National Primate Research Center, Seattle, United States, 3National Cancer Institute, Human Retrovirus Pathogenesis Section, Frederick, United States, 4National Cancer Institute, Human Retrovirus Section Vaccine Branch, Frederick, United States

Background: We have previously shown that in SIV-infected rhesus macaques undergoing antiretroviral therapy (ART), therapeutic DNA immunization protected ~50% of animals from viral rebound after discontinuing ART. To improve this approach, we are investigating a novel conserved elements (CE) therapeutic DNA vaccine which consists exclusively of CE sequences. We hypothesize that a CE DNA vaccine will achieve a more profound functional cure by forcing immune escape mutations in regions of the virus that would have the greatest impact on viral fitness. A question that must first be addressed is whether immunization with a vaccine expressing conserved, but generally subdominant epitopes, can induce responses against CE in the setting of an immunodominant response induced by infection. To investigate this question, we compared immunogenicity of a CE DNA vaccine to a DNA vaccine expressing whole SIV Gag in rhesus macaques chronically infected with SHIV, as well as the role of CE specific responses in long term viral control.

Methods: Two groups of rhesus macaques chronically infected with SHIV89.6P were immunized with either a traditional DNA vaccine expressing whole SIV Gag or an SIV CE DNA vaccine. An IFN-γ ELISpot assay was employed to map T cell responses induced in the blood and gut against the full SHIV proteome and the CE sequences. Intracellular cytokine staining was also used to assess functional quality of T cell responses directed against CE.

Results: Prior to immunization, both groups had similar responses to variable and immunodominant regions of Gag with little to no detectable responses to CE. Animals immunized with whole Gag exhibited no significant increase in responses against CE. In contrast, CE vaccinated animals developed a nearly ten-fold increase in IFNg and cytolytic T cell responses against CE.

Conclusions: These results illustrate that a CE DNA vaccine was able to overcome immunodominant responses associated with a viral infection and re-direct the cellular response toward increased targeting of the subdominant conserved viral sequences when compared to a traditional full length Gag DNA vaccine. These results support the feasibility of developing a therapeutic CE DNA vaccine to induce a functional cure against AIDS.

Under embargo until 14.30 on 22 July 2015
PE45
Immune response to sequences surrounding the 12 protease cleavage sites generated during ARV treatment improved CD4 counts of SIVmac251 infected rhesus monkeys

Luo M.1,2, Tang D.1, Pinto J.3, Nykoluk M.1, Lacap P.1, Czarnecki C.1, Tuff J.1, Capina R.1, Whitney J.1, Alonso M.3, Ball T.2,5, Kobinger G.2,6, Sandstrom P.5, Plummer F.2,7
1National Microbiology Laboratory, HIV Host Genetics/NHRL, Winnipeg, Canada, 2University of Manitoba, Medical Microbiology, Winnipeg, Canada, 3University of Santiago de Compostela, Santiago de Compostela, Spain, 4Harvard Medical School, Beth Israel Deconess Medical Center, Boston, United States, 5National Microbiology Laboratory, NHRL, Winnipeg, Canada, 6National Microbiology Laboratory, Special Pathogens, Winnipeg, Canada, 7National Microbiology Laboratory, Winnipeg, Canada

Background: Effective therapeutic vaccines used in combination of ARV to treat HIV infected patients can reduce drug induced toxicity, help to re-constitute immune system and achieve a functional cure. We conducted a pilot study to test the therapeutic effect of a novel HIV vaccine targeting the 12 protease cleavage sites in combination of ARV.

Methods: SIVmac251 infected rhesus monkeys were treated with a combination of FTC, PMPA and raltegravir for 49 days. Seven days after ARV initiation the monkeys in the treatment group received rVSVpcs (i.m.). Three additional therapeutic treatment with rVSVpcs (i.m.)/NANOpcs(i.n.), NANOpcs(i.n), and NANOpcs(i.n.) were carried out with 2-week intervals. ARV treatment was stopped after 49 days and viral load, CD4/CD8 counts, antibody and T cell response to PCS peptides and pooled Gag and Env peptide were analyzed.

Results: ARV treatment suppressed viral load of all macaques, but only the viral load of 6 out of 11 macaques was suppressed to non-detectable level during the treatment/ARV period. However, even with the short duration of ARV treatment and incomplete viral load suppression, the immune responses to PCS peptides were generated after 4 therapeutic treatments. The CD4 counts of PCS vaccine treated macaques were significantly improved after 35 days and 49 days of ARV treatment (p= 0.027 and 0.044), whereas there is no significant improvement in CD4 counts of monkeys only received ARV treatment despite the viral load suppression.

Conclusions: Our study showed that new immune response to PCS peptides can be generated even with incomplete viral load suppression after a short period ARV treatment. The combination of PCS vaccine treatment and ARV generated new immune response to PCS peptides, improved CD4 counts of SIVmac251 infected monkeys and can be used to improve patient care to achieve a functional cure.
Safety and immunogenicity of ChAd.HIVconsv and MVA.HIVconsv therapeutic vaccines in a cohort of early treated HIV-1 infected individuals

Mothe B.1,2,3, Manzardo C.4, Coll P.1,2, Cobarsi P.2, Sanchez-Bernabeu A.1, Escrig R.3, Perez-Alvarez N.2,3, Miró J.M.4, Dorrell L.6, Clotet B.1,2,3, Hanke T.7, Brander C.1,3,8, BCN01 Study Group

1IrsiCaixa AIDS Research Institute - HIVACAT, Badalona, Spain, 2Fundació Lluita contra la Sida, Hospital Germans Trias i Pujol, Badalona, Spain, 3UVic-UCC, Vic, Spain, 4Hospital Clinic-IDIBAPS, Barcelona, Spain, 5Technical University of Catalonia, Barcelona, Spain, 6John Radcliffe Hospital, Oxford, United Kingdom, 7The Jenner Institute, Oxford, United Kingdom, 8ICREA, Barcelona, Spain

Background: T-cell vaccines targeting the most conserved regions of the HIV-1 proteome may be required for the elimination of the latent viral reservoir. HIVconsv vaccines vectored by chimpanzee adenovirus (ChAdV63) and modified vaccinia virus Ankara (MVA) have shown to induce high levels of effector T cells in healthy individuals (HIVCORE02 trial). BCN01 (NCT01712425) is a phase I study to evaluate the safety and immunogenicity of ChAdV63 and MVA.HIV-consv vaccines in early-treated HIV-1 infected individuals

Methods: 24 individuals identified with recent HIV infection (< 6m from acquisition) who initiated Tenofovir/Emtricitabine/Raltegravir within 1 week after diagnosis, received an intramuscular ChAdV63.HIVconsv (5x10^{10}vp) vaccination after 6 months under cART. Participants were given an MVA.HIVconsv booster immunization (2x10^{8}pfu) 24 or 8 weeks afterwards and were followed for 6 months. Local and systemic events were recorded for a minimum of 7 days following each immunization. Immunogenicity to the vaccine insert and the rest of the HIV-1 proteome was assessed by IFNγ ELISPOT.

Results: Local and systemic events after vaccination occurred in 22/24 individuals, mostly severity grade 1-2 and transiently (48 hours). Local pain was more often reported with MVA than ChAdV63 vaccination. Responses to conserved regions before cART initiation were only observed in 4 individuals and diminished significantly after achieving viral supression. All participants significantly increased T-cell responses that targeted the vaccine insert, with a peak 1-4 weeks after MVA vaccination (median of 1,015 SFC/106 PBMC, range 140-6,805, p=0.0003, Wilcoxon t-test compared to baseline). Over vaccination period, no unspecific expansion of T cells targeting HIV-1 regions outside HIVconsv insert or CEF was noted, allowing for an optimal focusing of T-cell responses on conserved regions (48% of total HIV immune response being HIVconsv-specific 4 weeks after MVA vaccination). Among vaccinees, no significant differences in peak immunogenicity was observed between short and long prime/boost regimen.

Conclusions: ChAd.HIVconsv and MVA.HIVconsv was a safe strategy to shift pre-existing immune response towards conserved, vaccine-encoded regions of HIV in a cohort of early-treated individuals and may set the stage for successful subsequent of cure strategies.

Under embargo until 14.30 on 22 July 2015
PE47
Development of a latency reversing activator vaccine (ACT-VEC) platform for HIV-1 cure therapy

Mann J.F.S.1, Biru T.2, Wille R.T.2, Klein K.1, Arts E.J.1
1University of Western Ontario, Microbiology and Immunology, London, Canada, 2Case Western Reserve University, Medicine, Cleveland, United States

Background: HIV-1 persists within cellular reservoirs as a transcriptionally silent provirus, creating a significant roadblock to cure research. Numerous promising therapeutic and pharmacological interventions are currently being evaluated; however to date none have resulted in reservoir eradication. We have designed an activator vaccine (ACT-VEC), using autologous derived VLPs, which target the resting CD4 T cell reservoir, inducing latency reversal. We describe the safeguards incorporated into our VLPs as well as preliminary data from our in vitro latency reversal studies.

Methods: Plasmids used in these studies were derived from the laboratory strain NL4-3, envC3 1086 and patient derived HIV-1 inserted into the pRECΔgag-U3 VLP-vector. ACT-VEC were generated with deleted (Δ) 5’LTR, AAH>RRK integrase mutation and deletions within the RNA packaging element (ΔSL3). VLPs were then created by HEK293T transfection. Resulting VLPs were assessed by RT-PCR for RNA content and for the presence of viral proteins by western blot. VLPs were co-cultured with autologous patient derived DCs and then used to activate autologous CD4 T cells from PBMC. An IFN-γ ELISpot was used to quantify virus specific T cells, p24 ELISA to measure viral latency reversal, and 454 deep sequencing to characterize HIV resulting from latency reversal and compare to viral DNA isolated from PBMC.

Results: Here we show our ACT-VEC VLPs have reduced HIV-1 RNA packaging (up to 221-fold), while having no impact on viral protein production. This along with mutations in Integrase and ΔSL3 rendered our ACT-VEC incapable of reverse transcription, integration, or RNA packaging. Preliminary studies involving deep sequence analysis revealed ACT-VEC are genetically diverse and identical to virus generated by our latency reversal assays. Significantly, autologous ACT-VEC were able to stimulate 30-fold more HIV RNA from infected T cells than Flu/Tet/CMV recall antigens and more than NL4-3 controls. Our latency reversal studies showed ACT-VEC outperform clinically relevant compounds such as Romidepsin and Vorinostat.

Conclusions: Here we clearly demonstrate that our novel ACT-VEC formulations represent a safe vaccine platform for use as a therapeutic intervention and that ACT-VEC may signify a promising strategy to purge the latent viral reservoir and facilitate cure.

Under embargo until 14.30 on 21 July 2015
PE48

Broadly specific, cytolytic T cell responses and lower inflammatory responses correlate with durable viral remission following therapeutic DNA vaccination in SIV-infected macaques

Fuller D.1,2, Narendran A.3, Rajakumar P.1, Che J.4, Yager E.3, Stagmar C.2, Murphey-Corb M.4
1Deborah L Fuller, University of Washington, Seattle, United States, 2Washington National Primate Research Center, AIDS Division, Seattle, United States, 3Albany Medical College, Albany, United States, 4University of Pittsburgh, Pittsburgh, United States

**Background:** We previously reported (DOI: 10.1371/journal.pone.0033715) that an adjuvanted DNA vaccine that stimulated mucosal CD8+ T cell responses in the gut of SIV-infected macaques during antiretroviral drug therapy (ART) induced 3 different virological outcomes: Viral rebound within 6 months after stopping ART (5/14 vaccinated animals and 6 controls), protection from viral rebound for 12-18 months after withdrawing drugs (5/14 animals) or no detectable virus (4/14 animals) for over 30 months (duration of the study) after stopping ART.

**Methods:** At study end, macaques were necropsied to determine the impact of vaccination on residual virus in the gut and lymphoid tissues. To define what immune responses contributed to long-term viral control, lymphocytes were isolated from blood and gut tissues and T cell responses and inflammatory cytokines in the blood and gut were measured by ICS, ELISPOT and cytometric bead array. Results in macaques that had no detectable virus or exhibited a significant delay in viral rebound after stopping ART were compared to macaques that exhibited immediate viral rebound within 6 months after stopping ART.

**Results:** The 4 macaques with no detectable virus in the blood had detectable viral RNA and/or DNA in at least one lymph node or in gut tissues demonstrating the vaccines substantially reduced residual virus but did not clear the virus. Animals that exhibited delayed viral rebound or no viral rebound had a higher frequency of CD8+ T cells with cytolytic effector function, higher CD4+ T cell proliferation, and broadly specific mucosal SIV-specific CD8+ T cell response targeting more conserved viral sequences in Gag when compared to animals that rebounded within 6 months after stopping ART. In addition, lymphocytes isolated from macaques that exhibited delayed or no viral rebound post-ART expressed lower levels of the inflammatory cytokines (TNF-α, IL-6) prior to stopping ART when compared to macaques that exhibited immediate viral rebound within 6 months post-ART.

**Conclusions:** These results show that immunotherapuetics that can broaden virus-specific T cell responses against more conserved viral sequences and at the same time, reduce inflammation during HAART may be an effective approach to achieve durable viral remission.
PE49
Crispr/Cas9 gene editing eradicates latent and protects cells against new HIV-1 infection

Kaminski R., Hu W., Zhang Y., Karn J., Khalili K.
1Temple University, Neuroscience, Philadelphia, United States, 2Case Western Reserve, Cleveland, United States

**Background:** A sterilizing cure for HIV-1/AIDS requires a strategy that eliminates all or at least some critical regions of the HIV-1 genome including the promoter positioned within the 5’ LTR of the viral genome from cells serving as a stable reservoir for HIV-1, i.e. resting CD4+ T-lymphocytes, macrophages, and brain microglia, with no adverse impact on the host cells.

**Methods:** We have tailored CRISPR/Cas9 gene editing by bioinformatic screening, Surveyor assay, whole genome sequencing, and have successfully developed a series of guide RNAs (gRNAs) that, in complex with Cas9 nuclease, effectively and safely eliminate integrated copies of HIV-1 proviral DNA in several human cell culture models. We assessed the impact of our gene editing strategy on viral transcription and replication by measuring the level of a GFP reporter and viral p24, upon reactivation of virus from the latent stage by treatment with PMA and TSA.

**Results:** We demonstrated inactivation of HIV-1 gene expression and replication in latently infected T-lymphocytes and promonocytic human cell lines as well as microglial cells upon excising the proviral DNA fragment corresponding to the entire coding sequence of HIV-1 spanning the 5’ to 3’ LTRs from the host chromosome by the CRISPR/Cas9 approach. Further, we demonstrate that the presence of LTR-specific multiplex of guide RNAs in cells expressing Cas9 acts as an efficient inhibitor blocking new HIV-1 infection.

Conclusions: Our findings suggest that the strategy involving the newly developed CRISPR/Cas9 serves as a promising platform that can be advanced for eradication of HIV-1 and a cure for AIDS.

Under embargo until 14.30 on 21 July 2015
**PE50**

**High rates of non-reactive HIV serology after antiretroviral treatment initiated in acute HIV infection**

Fletcher J.L.K.†, Pinyakorn S.‡, de Souza M.¶, Akapirat S.¶, Trichavaroj R.¶, Pankam T.¶, Kroon E.†, Colby D.†, Pruksakaew P.†, Suttichom D.†, Kim J.H.†, Phanuphak P.¶, Phanuphak N.¶, Ananworanich J.¶, The SEARCH010/RV254 Study Group

†SEARCH, The Thai Red Cross AIDS Research Centre, Bangkok, Thailand, ‡U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, United States, ¶Armed Forces Research Institute of Medical Sciences - United States Component, Department of Retrovirology, Bangkok, Thailand, 5The Thai Red Cross Anonymous Clinic, Thai Red Cross AIDS Research Centre, Bangkok, Thailand, ⁶Chulalongkorn University, Department of Medicine, Faculty of Medicine, Bangkok, Thailand, ⁷Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, United States

**Background:** Non-reactive HIV serology may be a marker of low HIV viral burden. We examined the evolution of HIV antibody in a cohort of individuals treated during acute HIV infection (AHI).

**Methods:** Between April 2009 and December 2014, adults attending voluntary HIV testing in Bangkok, Thailand, were screened for AHI, by either pooled nucleic acid testing (NAT) of 4th generation immunoassay (4G IA) non-reactive samples or by 3rd (3G) or 2nd generation (2G) enzyme immunoassay (EIA) of 4G IA reactive samples. Immediate antiretroviral therapy (ART) was offered. Western blot and p24 quantification were performed for Fiebig staging. HIV serology at baseline, weeks 12 and 24 were performed.

**Results:** 233 Thai adults were enrolled from 130,164 samples screened; 3 individuals did not initiate ART and were excluded from analysis. The median age of the volunteers was 27 years and 95% were male. Median time from history of HIV exposure to enrollment was 18 days and median time from enrollment to ART initiation was 1 day. Of 207 baseline 2G EIA non-reactive subjects, results were available for 150 at week 12 and 135 at week 24 (Table 1). At week 12, 34% were non-reactive by 2G, 3% by 3G and 20% by 4G IA; at week 24, 39% were non-reactive by 2G, 5% by 3G and 18% by 4G. Baseline HIV RNA < 5 log10 copies/ml (p=0.02), CD4 count > 350 cells/µL (p=0.01) and Fiebig stage 1 or 2 (p=0.03) were predictive of non-reactive 2G EIA at week 24. Lower AUC0-24wk for HIV RNA was also associated with non-reactive 2G EIA at week 24 (p=< 0.001, Figure 1).

Seroreversion was uncommon. 1 of 23 individuals with reactive 2G EIA at baseline was non-reactive at week 24; 11 of 207 demonstrated transient 2G EIA reactivity at week 12.

<table>
<thead>
<tr>
<th>Non-reactivity to HIV enzyme immunoassay [N(%)]</th>
<th>Baseline (N=207)</th>
<th>Week 12 (N=150)</th>
<th>Week 24 (N=135)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd generation EIA</td>
<td>207 (100)</td>
<td>51 (34)*</td>
<td>53 (39)*</td>
</tr>
<tr>
<td>3rd generation EIA</td>
<td>99 (48)</td>
<td>5 (3)*</td>
<td>7 (5)*</td>
</tr>
<tr>
<td>4th generation IA</td>
<td>43 (21)</td>
<td>30 (20)</td>
<td>24 (18)</td>
</tr>
</tbody>
</table>

* McNemar’s test, p<0.001, compared to baseline [Note: No significant difference between week 12 and week 24]

[Table 1: Non-reactivity to enzyme immunoassay]
Conclusions: Approximately 40% of individuals who initiated treatment in AHI maintained non-reactivity to 2G EIA after 24 weeks of ART. Rapid ART initiation and HIV RNA decline as well as low HIV RNA and high CD4 at baseline predicted subsequent serological nonreactivity. HIV serologic non-reactivity is likely due to low viral burden, further supporting the benefits of early initiation of ART.

Under embargo until 14.30 on 22 July 2015
Early initiation rather than prolonged duration of antiretroviral therapy in HIV infection contributes to reducing CD8 T-cell elevation: Relevance for clinical outcome

W. Cao¹²³, V. Mehraj¹², B. Trotter⁴, J.-G. Baril⁵, R. Leblanc²⁴, B. Lebouche², J. Cox², C. Tremblay⁷⁸, J.-P. Routy¹²⁹
¹Research Institute, McGill University Health Center, Montreal, Canada, ²Chronic Viral Illness Service, McGill University Health Center, Montreal, Canada, ³Peking Union Medical College Hospital, Department of Infectious Diseases, Beijing, China, ⁴l’Actuel Medical Clinic, Montreal, Canada, ⁵Clinique Médicale Quartier Latin, Montreal, Canada, ⁶Clinique Médicale OPUS, Montreal, Canada, ⁷CHUM Research Centre, Montreal, Canada, ⁸University of Montreal, Department of Microbiology and Immunology, Montreal, Canada, ⁹Division of Hematology, McGill University Health Centre, Montreal, Canada

Background: HIV infection is featured by profound immune dysfunction and skewed T-cell homeostasis. Elevation of CD8 T-cells occurs during primary HIV infection (PHI) and persists after long-term antiretroviral therapy (ART), which has been associated with increased risk of non-AIDS-related morbidity and mortality independently of CD4 T-cell recovery. We examined factors associated with trajectories of CD8 T-cell counts in early treated or untreated PHI and assessed influence of early vs. chronic ART initiation on CD8 T-cell elevation over time.

Methods: From 1996 to 2012, a total of 280 individuals (95.5% male, 92.5% Caucasians) were enrolled in the Montreal PHI study. Plasma viral load (VL), CD4 and CD8 T-cells were measured at each study visit. We also assessed 266 age and gender-matched HIV-infected individuals from McGill University Health Centre, Montreal, who initiated ART during chronic infection and have maintained undetectable VLs for at least one year. Another 40 uninfected individuals were included as controls. Regression analyses were performed.

Results: 251 PHI individuals were longitudinally assessed, where 84 started ART before 6 months of infection (early ART), 49 between 6 and 24 months (delayed ART) and 118 remained untreated as per physician/patient decision. Baseline characteristics and stratified CD8 T-cell counts were summarized in Table 1. At first PHI visit, CD8 T-cell counts were significantly associated with duration of infection (p=0.019), VLs (p<0.001) and CD4 T-cell counts (p<0.001). Early ART group achieved a marked decrease in CD8 T-cell counts from 797 to 588 cells/μl over 2 years (p<0.001), which remained elevated compared to uninfected controls (median 376 cells/μl, p<0.001), but significantly lower than untreated (834 cells/μl, p=0.004) and chronic patients after a median of 8-year-ART (801 cells/μl, p=0.004). Early ART group displayed more CD4 T-cell recovery than the delayed group. However, overtime CD8 T-cell counts remained similar in the 2 groups.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
<th>Stratification</th>
<th>No of participants</th>
<th>Median CD8 T-cell count, cells/µl</th>
<th>P value Univariate</th>
<th>P value Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td>266</td>
<td>800 (598-1265)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>36.1±9.4</td>
<td>&lt;50/≥50</td>
<td>247/19</td>
<td>826/710</td>
<td>0.771</td>
<td>0.991</td>
</tr>
<tr>
<td>Gender</td>
<td>Male/Female</td>
<td>254/12</td>
<td>824/715</td>
<td></td>
<td>0.641</td>
<td>0.308</td>
</tr>
<tr>
<td>Route of infection</td>
<td>MSM/Hetero/IDU</td>
<td>207/21/38</td>
<td>853/660/790</td>
<td></td>
<td>0.148</td>
<td>0.824</td>
</tr>
<tr>
<td>Time of infection, days</td>
<td>82(56-121)</td>
<td>Fiebig II-III/IV/V-VI</td>
<td>40/81/145</td>
<td>935/810/787</td>
<td>0.118</td>
<td>0.019</td>
</tr>
<tr>
<td>HIV RNA load, log copies/ml</td>
<td>4.59±1.07</td>
<td>&lt;3/3-5/&gt;5</td>
<td>19/152/95</td>
<td>650/782/1140</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4 T-cell count, cells/µl</td>
<td>500(380-658)</td>
<td>&lt;500/≥ 500</td>
<td>124/142</td>
<td>740/880</td>
<td>0.005</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusions: ART initiated in early HIV infection was associated with better resolution of CD8 T-cell elevation, while long-term ART initiated in chronic phase showed limited benefit in CD8 T-cell decrease. In addition to CD4 T-cell recovery, early ART may further contribute to reducing risk of non-AIDS events by alleviating CD8 T-cell elevation.
HIV reservoirs in semen at the time of Primary infection

A. Chéret1,2, J. Heitzmann3, A. Mélard2,4, C. Durier5, L. David4, J.-M. Chennebault6, P. Philibert7, L. Meyer3,8, C. Rouzioux2,4, ANRS-147 OPTIPRIM Study Group

1Bicêtre Hospital, AP-HP, Internal Medicine Unit, Le Kremlin-Bicêtre, France, 2Paris Descartes University, EA 7327, Paris, France, 3INSERM SC10-US19, Villejuif, France, 4AP-HP, Necker Hospital, Virology Laboratory, Paris, France, 5INSERM SC10-US19, Villejuif, France, 6Angers Hospital, Infectious Diseases Department, Angers, France, 7Ambroise Paré Hospital, Internal Medicine Department, Marseille, France, 8Université Paris 11, Faculté de Médecine Paris-Sud, INSERM, CESP UI018, Bicêtre, France

Background: Primary-HIV-Infection (PHI) is a high-risk period for viral transmission. The objective of this work was to study blood and seminal reservoir virological markers and the impact of early treatment in patients with Primary HIV Infection.

Methods: Patients from the ANRS-147-OPTIPRIM randomized trial received two years of early-cART. The 21 patients who also accepted to provide semen samples were recruited in this HIV reservoir substudy. Blood and seminal samples were collected at inclusion and month 24; total cell-associated-HIV-DNA and HIV-RNA were quantified in blood and in semen cells and seminal plasma (Biocentric, Bandol, France). Spearman correlation tests were performed.

Results: Twenty-one patients were enrolled (median age: 36 years, time from estimated date of infection: 33 days), 20 were symptomatic and 8 presented during acute infection (WB ≤1 Ab). At enrollment, median CD4 T-cell count was 465 cells/mm3 [min-max: 163-1116]; blood-HIV-RNA was correlated with CD4 count (r=-0.54, p=0.017), CD4/CD8 ratio (r=-0.61, p=0.005). Median HIV-RNA was significantly higher in blood (5.39 log10 cp/mL [4.07-7.00]) than semen samples (4.22 log10 cp/mL [2.57-6.27]) and no correlation was observed between HIV-RNA in blood and semen. We found significant correlations between semen-HIV-RNA and CD4+ T cells (r=-0.54, p=0.018), CD8+T cells (r=-0.54, p=0.018). Blood-HIV-DNA was 3.59 log10 cp/106PBMC [2.78-4.5] and did not correlate with semen-HIV-DNA (10/19 had detectable HIV-DNA in semen).

Among 8 patients presenting acute infection, semen-HIV-RNA was correlated with blood-HIV-RNA (r=0.81, p=0.015), CD4 count (r=-0.98, p<0.0001), CD4/CD8 ratio (r=-0.85, p=0.0075).

At M24, blood-and semen HIV-RNA levels became < threshold of detection. All positive semen-HIV-DNA decreased to undetectable level in all but one patient who reported use of recreational drugs at that time point (which might explain this positive result).

Conclusions: This is the first evidence of HIV-reservoir cells in semen of patients with acute infection, showing that levels are linked with the immunosuppression severity. Infected cells in semen represent a factor associated with an increased risk of HIV transmission via cell to cell transmission. Early treatment allows purging viral particles but also infected cells to limit viral transmission.
PE53 LB
CD4/CD8 ratio at ART initiation as a predictor of viral rebound following interruption of ART initiated in primary HIV infection

J. Thornhill¹, J. Inshaw³, P. Kaleebu³, D. Cooper³, G. Ramjee³, M. Schechter³, G. Tambussi⁷, J. Fox³, J.M. Miro⁹, J. Weber¹, A. Babiker⁲, J. Frater⁰, K. Porter³, S. Fidler¹
¹Imperial College, Department of Medicine, London, United Kingdom, ²MRC Clinical Trials Unit at UCL, Institute of Clinical Trials & Methodology, London, United Kingdom, ³Medical Research Council, Uganda Virus Research Institute, Entebbe, Uganda, ⁴University of New South Wales, Kirby Institute, Sydney, Australia, ⁵Medical Research Council, HIV Prevention Unit, Durban, South Africa, ⁶Projeto Praca Onze, Universidade Federal do Rio de Janeiro, Hospital Escola Sao Francisco de Assis, Rio de Janeiro, Brazil, ⁷Ospedale San Raffaele, Division of Infectious Diseases, Milan, Italy, ⁸Kings College London, Guys and St Thomas’ NHS Trust, London, London, United Kingdom, ⁹University of Barcelona, Hospital Clinic, Barcelona, Spain, ¹⁰University of Oxford, Oxford, United Kingdom

Background: Virological post-treatment control (PTC) has been described among individuals commencing ART in primary HIV infection (PHI). Factors which predict likelihood of PTC are required to inform treatment interruption (TI) studies. Preserved immunological function plays a key role in viral control amongst Elite controllers. We investigated the association between CD4/CD8 at ART initiation with time to rebound amongst individuals initiating short-term ART during PHI.

Methods: Time to event analyses and Cox proportional hazards models were used to investigate effect of CD4/CD8 at time of ART initiation and TI with time from ART interruption to virological rebound (>400c/mL), censoring at ART re-initiation, and adjusting for: sex, age, exposure group, enrolment site, HIV Viral Load (VL) at ART initiation, time from seroconversion to ART initiation, and ART duration. Data from two cohorts of treated HIV-seroconverters; the UKR HIV Seroconverters and the SPARTAC RCT, were used.

Results: Of 206 individuals, 142 male, median age 34 years, median time from seroconversion to ART initiation 84 (IQR 57-106) days and median ART duration 3.37 months, 202 (98%) experienced viral rebound. Median (95%CI) time to rebound was 31 (28,71) days, with 4 not experiencing a rebound by 3 years.

In unadjusted analyses, higher ART initiation CD4/CD8 (HR [95% CI] =0.64 [0.44, 0.91], p=0.015), HIV VL (HR[95% CI]=1.27 [1.11,1.46] p=0.001) and longer duration of ART (HR [95% CI] =0.97 [0.93, 0.99], p=0.032) were associated with longer time to rebound. TI CD4/CD8 was not. On adjusting for all other factors, there was no evidence of an independent effect of CD4/CD8 at ART initiation (p=0.39), although some evidence that those with a ratio ≥ 1.2 (HR [95%CI] = 0.59 [0.345,0.997],p=0.049), compared with < 1.2, experienced longer time to rebound. Higher VL at time of starting ART (p=0.017), and longer duration of ART (p=0.045) were associated with longer time to rebound.

Conclusions: A CD4/CD8 ratio ≥ 1.2 at ART initiation may be predictive of post treatment control. Initiation of ART before disruption of immune homeostasis below a threshold may be important in controlling viraemia during ART cessation; CD4/CD8 ratio warrants further evaluation in cohorts with longer duration of treatment where PTC would be anticipated.
### Unadjusted Analyses

<table>
<thead>
<tr>
<th>Factor</th>
<th>HR</th>
<th>95.0% CI Lower</th>
<th>95.0% CI Upper</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>.760</td>
<td>.556</td>
<td>1.039</td>
<td>.085</td>
</tr>
<tr>
<td>Time from seroconversion to ART Initiation</td>
<td>.999</td>
<td>.998</td>
<td>1.000</td>
<td>.189</td>
</tr>
<tr>
<td>ART Initiation HIV Viral Load (Log cpm)</td>
<td>1.274</td>
<td>1.111</td>
<td>1.460</td>
<td>.001</td>
</tr>
<tr>
<td>CD4/CD8 at ART initiation</td>
<td>.636</td>
<td>.443</td>
<td>.914</td>
<td>.015</td>
</tr>
<tr>
<td>CD4/CD8 at Treatment Interruption</td>
<td>.764</td>
<td>.579</td>
<td>1.009</td>
<td>.058</td>
</tr>
<tr>
<td>Duration on ART (months)</td>
<td>0.965</td>
<td>.933</td>
<td>.997</td>
<td>.032</td>
</tr>
<tr>
<td>Year of HIV Seroconversion</td>
<td>.965</td>
<td>.897</td>
<td>1.039</td>
<td>.347</td>
</tr>
<tr>
<td>CD4 ≥ or &lt; 900 at TI</td>
<td>1.141</td>
<td>.831</td>
<td>1.567</td>
<td>.415</td>
</tr>
<tr>
<td>Age Category</td>
<td>.964</td>
<td>.856</td>
<td>1.085</td>
<td>.539</td>
</tr>
<tr>
<td>Exposure Category MSM</td>
<td>2.769</td>
<td>.680</td>
<td>11.278</td>
<td>.155</td>
</tr>
<tr>
<td>Enrolment from an African site</td>
<td>1.416</td>
<td>1.013</td>
<td>1.980</td>
<td>.042</td>
</tr>
<tr>
<td>CD4/CD8 at TI ≥ 1.2 compared to &lt;1.2</td>
<td>.721</td>
<td>.534</td>
<td>.975</td>
<td>.034</td>
</tr>
<tr>
<td>ART initiation CD4/CD8 ≥1.2 or &lt;1.2</td>
<td>.430</td>
<td>.265</td>
<td>.697</td>
<td>.001</td>
</tr>
</tbody>
</table>

### Multivariable Analyses

<table>
<thead>
<tr>
<th>Factor</th>
<th>HR</th>
<th>95.0% CI Lower</th>
<th>95.0% CI Upper</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>.721</td>
<td>.265</td>
<td>1.96</td>
<td>.522</td>
</tr>
<tr>
<td>Enrolment from an African site</td>
<td>1.625</td>
<td>.802</td>
<td>3.292</td>
<td>.178</td>
</tr>
<tr>
<td>Time from seroconversion to ART Initiation</td>
<td>1.000</td>
<td>.999</td>
<td>1.001</td>
<td>.601</td>
</tr>
<tr>
<td>ART Initiation HIV Viral Load (Log cpm)</td>
<td>1.221</td>
<td>1.036</td>
<td>1.439</td>
<td>.017</td>
</tr>
<tr>
<td>Duration on ART (months)</td>
<td>.962</td>
<td>.927</td>
<td>.999</td>
<td>.045</td>
</tr>
<tr>
<td>ART initiation CD4/CD8 ≥ 1.2 or &lt;1.2*</td>
<td>.586</td>
<td>.345</td>
<td>.997</td>
<td>.049</td>
</tr>
</tbody>
</table>

Multivariable model adjusted for sex, age, exposure, enrolment from an African site, time from seroconversion to ART Initiation, HIV Viral Load (Log), Duration on ART, and Time from both viral load and CD4/CD8 reading to ART Initiation.

*Model for CD4/CD8 ratio as a continuous variable was not significant, a poorer model fit (as measured by AIC) and is not shown.
p<.05 shown in bold.

[Cox Model: Factors associated with rebound]
Time To Rebound - CD4/CD8 ≥ 1.2 and <1.2
Long-term non-progressors and elite controllers

PE54

Ultrastop: Is remission achievable in HIV-1 patients with low HIV DNA reservoir?

R. Calin1,2,3, S. Lambert-Niclot1,2,3, C. Hamimi1,2, Y. Dudoit1,2,3, L. Assoumou1,2,3, R. Tubiana1,2,3, V. Calvez2,3,4, B. Autran3,6, D. Costagliola2,3, C. Katlama1,2,3

1AP-HP, Department of Infectious Diseases, Pitié-Salpêtrière University Hospital, Paris, France, 2Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, Institut Pierre Louis d’Épidémiologie et de Santé Publique, Paris, France, 3INSERM, UMR_S 1136, Institut Pierre Louis d’Épidémiologie et de Santé Publique, Paris, France, 4AP-HP, Virology Department, Pitié-Salpêtrière University Hospital, Paris, France, 5Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1135, Institut Pierre Louis d’Épidémiologie et de Santé Publique, Immunology Department, Paris, France, 6Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, Institut Pierre Louis d’Épidémiologie et de Santé Publique, Immunology Department, Paris, France

Background: Viral remission is observed in elite controllers and post early-treatment controllers (PTCs). All share a good immune status and extremely low blood total cell-associated HIV-DNA levels. ULTRASTOP investigates whether HIV remission after ART discontinuation can be achieved in long-term HIV chronically-infected patients with good immunological status and low-level DNA.

Methods: This proof-of-concept study was designed to involve 3 cohorts of 5 patients (pts) with pVL < 50 copies (cp)/mL for >2 years on ART, CD4 > 500/mm3, CD4/CD8 > 0.9, CD4 nadir > 300/mm3 and HIV-DNA < 100 cp/106 PBMCs, selected for treatment interruption. Ultrasensitive pVL, CD4, triplicate HIV-DNA were measured at D0, W2, W4, and every 4 weeks off-ART until W48 and at W4, W12 and W24 after ART resumption (RxR). Treatment was resumed in case of pVL rebound > 400 cp/mL or CD4 < 400 cells or HIV-related clinical event. The primary endpoint was the percentage of patients who did not reach RxR criteria at W24. Enrolment in cohort 2 started, when 1/5 pts remained in success at W8. Cohort 3 did not start.

Results: Ten patients were enrolled in cohort 1, then 2, with median (min-max) duration of ART of 5.3 years (3.0-15.5), viral suppression 4.9 years (2.9-8.3), CD4 nadir 495/mm3 (330-739), baseline CD4 1118/mm3 (608-1494), CD4/CD8 2.1 (1.4-2.6), HIV-DNA 66 cp/106 PBMC (< 66-80). One patient remained off-ART at W40. Viral rebound occurred in 9/10 pts: W2 (2pts), W4 (6pts) and W12 (1pt) with CD4 counts of 745/mm3 (578-1438), pVL was resuppressed on cART (< 50 cp/ml) at W4 (8pts) and W12 (1pt) with a median of 835 CD4/mm3 (705-1326), CD4/CD8 ratio of 1.3 (1.1-2.1). In all patients from cohort 1 (cohort 2 on-going), HIV-DNA after increasing at time of rebound, returned to baseline values within 12 weeks following RxR.
Conclusions: Despite excellent immuno-virological characteristics apparently close to those of PTCs treated at primary infection, chronically-infected patients had viral rebound in a short delay. Extensive analyses of the viral and cellular dynamics are on-going. Importantly, rapid kinetics of HIV-DNA levels after ART discontinuation and RxR with return of each patient to their baseline status, suggests that the intervention with this study design has not been deleterious.
Timing of therapy initiation

PE55
Initiation of Antiretroviral Therapy at high CD4 cell counts is associated with Increased Adherence, Viral Suppression, and Decreased HIV Drug Resistance in British Columbia, Canada.

British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada

Background: Early initiation of combined antiretroviral therapy (cART) has been shown to have a consistent beneficial impact on morbidity and mortality. Conversely, there is limited research investigating the possible mechanisms of how starting cART at higher CD4s decreases mortality. Therefore, we conducted this present study to investigate the association between initiating cART at different CD4 cutoffs with: short- and long-term achievement of viral suppression; the emergence of drug resistance and of an AIDS-defining illness (ADI); long-term treatment adherence; and all-cause mortality.

Methods: This retrospective cohort study included 4120 antiretroviral naïve patients who initiated cART between 2000 and 2012. Patients were followed until 2013, death or until the last contact date (varied by outcome). The main exposure variable included the interaction between the period of cART initiation (2000-2006 and 2007-2012) and CD4 at cART initiation categorized as < 500 versus ≥500 cells/mm3. We considered both baseline and longitudinal covariates. We fitted different multivariable models using cross-sectional and longitudinal statistical methods, depending on the outcome.

Results: Initiation of cART at CD4s ≥500 cells/mm3 was associated with several positive treatment outcomes, suggesting that these outcomes are likely to play an important role in explaining the positive impact of early cART initiation on mortality. Patients who initiated cART with a CD4 ≥500 cells/mm3 in 2007-2012 had an increased likelihood of achieving viral suppression at nine months and of maintaining an adherence level ≥95% over time, and the lowest probability of developing any resistance and an ADI during follow-up. Patients who initiated cART with a CD4 ≥500 cells/mm3 in 2007-2012 were not the ones with the highest likelihood of maintaining viral suppression over time, most likely due to viral load blips that have happened during the follow-up time. Despite these viral load blips resulting in a perceived lower probability of maintaining viral suppression over time, we showed that this outcome did not negatively influence the mortality of these patients.

Conclusions: Our results should alleviate some of the concerns clinicians may have when initiating cART in people with CD4s ≥500 cells/mm3, as recommended by current guidelines, particularly where free access to modern cART and related monitoring is available.
PE56
Long-term antiretroviral therapy limits the HIV-1 reservoir size as compared to later treatment initiation but not to levels found in long-term non-progressors

Malatinkova E.1, De Spiegelaere W.1, Bonczkowski P.1, Kiselinova M.1, Vervisch K.1, Trypsteen W.1, Johnson M.2, de Loose D.1, Kinloch S.2, Vandekerckhove L.1
1Ghent University and University Hospital Ghent, HIV Translational Research Unit, Department of Internal Medicine, Faculty of Medicine and Health Sciences, Ghent, Belgium, 2University College London, Royal Free Hospital, Division of Infection and Immunity, Royal Free Campus, London, United Kingdom, 3University Hospital Ghent, Ghent, Belgium

Background: Early initiation of long-term antiretroviral therapy (ART) may lead to viral control after treatment discontinuation. Recent evidence indicates that ART initiated within seroconversion limits the HIV-1 reservoir size. Insight into the reservoir in patients with different timings of ART as well as those who can control HIV-1 without therapy should further inform new treatment strategies.

Methods: A cross-sectional study of HIV-1 reservoir size (total and integrated HIV-1 DNA) and dynamics (2-LTR circles and cell-associated HIV-1 unspliced RNA (usRNA)) was performed in peripheral blood mononuclear cells (PBMCs) in 84 HIV-1 infected patients from 4 cohorts in 2 clinical centers (London, UK and Ghent, BE): long-term treated patients with ART initiated during seroconversion (SRCV on ART; n=25) or chronic infection (Chronic ART; n=32), long-term non-progressors (LTNP; n=17) and ART-naïve recent seroconverters (Recent SRCV; n=10). Total HIV-1 DNA, 2-LTR and usRNA were measured by ddPCR and integrated HIV-1 DNA by Alu-HIV PCR. Clinical parameters including time on ART and aviremia, CD4 count and CD4/CD8 ratio were collected.

Results: Median total HIV-1 DNA copies were: 92, 48, 137 and 1901 c/106 PBMCs in SRCV on ART, LTNP, Chronic ART and Recent SRCV, respectively. Significantly lower levels of total (p=0.041) and integrated HIV-1 DNA (p=0.003) were detected in early as compared to chronically treated patients, however these were higher than those found in LTNP (Fig.1a, 1b). Interestingly, similar levels of integrated HIV-1 DNA were found in Recent SRCV compared to the Chronic ART cohort (p=0.104), confirming very fast seeding of the reservoir (Fig 1b). Levels of usRNA were significantly lower in early compared to chronically treated cohort (p=0.007), indicating a lower transcriptional activity in early treated patients and similar to LTNP (p=0.615). Furthermore, early treated patients exhibited a higher CD4/ CD8 ratio compared to chronically treated patients (p=0.009), suggesting lower levels of residual immune activation.
**Conclusions:** Our data demonstrate that long-term early treated patients have smaller reservoir size as compared to patients treated during chronic infection, however not reaching levels found in LTNP. Interestingly, the reservoir dynamics in terms of 2-LTR and usRNA as well as the CD4/CD8 ratio in early treated patients are comparable to LTNP.

*Under embargo until 14.30 on 22 July 2015*
Ethical issues in clinical trials and treatment strategies

PE57

Proposed HIV cure research in South Africa: perspectives of HIV researchers, clinicians and advocates on the anticipated ethical challenges

K. Moodley1, T. Rossouw2, C. Staunton1, C. Colvin3
1Stellenbosch University, Centre for Medical Ethics and Law, Cape Town, South Africa. 2University of Pretoria, Immunology, Pretoria, South Africa. 3University of Cape Town, Public Health, Cape Town, South Africa

Background: South African health researchers and clinicians have been actively involved in HIV research for more than three decades. Despite challenges in HIV prevention research and treatment trials, the research community in South Africa is committed to future research in search of a “cure”. To date HIV research has focussed on prevention and treatment and associated ethical challenges. More recently the ethics of proposed HIV cure research has received attention: therapeutic misconception, risk-benefit ratio and study design. To date there has been no published empirical enquiry into key informant perspectives on HIV cure research in South Africa. This study was conducted to gain preliminary data to guide future phases of a larger multisite HIV cure project on ethical and social issues related to HIV cure.

Methods: A purposive sample of twelve key informants in academic institutions, HIV clinics and HIV research units in South Africa, was interviewed after obtaining informed consent. Recorded interviews were transcribed verbatim with concurrent thematic analysis. To establish data credibility we triangulated the perspectives of three groups of participants: researchers working in treatment and prevention, HIV clinicians, HIV activists (triangulation of sources). In addition, analyst triangulation occurred.

Results: Common themes emerged from in-depth interviews: HIV cure research has enhanced understanding of the pathogenesis of HIV, especially viral reservoirs. Insights about sterilizing and functional cures have parallels in oncology especially remission. Cure research should not replace or overshadow treatment and prevention research. Cure science is complex and must be translated for communities. Cure research should not be limited to the developed world. We should not create undue expectations/therapeutic misconception. The risks of cure research and treatment interruption must be acknowledged.

Conclusions: A holistic approach integrating biomedical treatment, prevention and cure research is critical with cognisance of social and ethical dimensions. Resource allocation to all domains is imperative. Community engagement to translate complex HIV cure science into lay conversations about cure to enhance consent processes is essential. We need research in Africa with capacity development and technology transfer. Knowledge sharing and collaboration with research scientists in treatment and prevention will accelerate progress towards cure.
The ethics of HIV cure clinical research among acutely infected adults: points for consideration

A.L. Gilbertson1,2, S. Rennie3, J. Tucker4

1University of North Carolina at Chapel Hill, Chapel Hill, United States, 2University of Oxford, School of Anthropology and Museum Ethnography, Oxford, United Kingdom, 3University of North Carolina at Chapel Hill, Social Medicine, Chapel Hill, United States, 4University of North Carolina at Chapel Hill, Division of Infectious Diseases, Chapel Hill, United States

Background: Individuals with acute HIV infection are increasingly targeted for participation in HIV cure clinical trials around the world. However, acute HIV infection may be a socially and emotionally tumultuous period, presenting special challenges when involving individuals in HIV cure research. The purpose of this study was to better understand the experiences of acutely infected individuals (AIs) in order to inform HIV cure research.

Methods: We examined the social hallmarks of acute HIV infection and explored ethical challenges in HIV clinical research in order to offer points to consider when developing guidelines for conducting HIV cure research. A comprehensive review of literature concerning experiences during acute infection and the ethics of clinical research within HIV treatment, prevention, and cure-related studies was conducted. Efforts were made to identify specific areas of concern for research involving AIs.

Results: We found that experiences during acute HIV infection may include confusion, guilt, anger, uncertainty, and/or other feelings that may complicate participation in clinical research. We identified four ethical domains of potential concern related to AIs:

(1) Identifying and building research cohorts;
(2) Recruitment and voluntary informed consent (including therapeutic misconception);
(3) Retention in clinical research;
(4) Concurrent/post-intervention care and researcher responsibilities.

Existing HIV research ethics guidelines focus on chronic rather than acute infections. We provide ethical points for consideration concerning each of these domains related to research with AIs.

Conclusions: Significant gaps in current knowledge exist concerning the recruitment and retention of AIs to HIV cure-related clinical trials, and necessitate further research. Our study suggests that the social context and personal dimensions of acute HIV infection introduce special ethical concerns that should be addressed during research design and ethical approval. The points of consideration identified may provide an initial foundation for ethical guidelines focused on research with AIs.
Therapeutic vaccine trials

PE59

HIV-1 reservoir dynamics after vaccination and antiretroviral therapy interruption are driven by dendritic cell-vaccine induced T-cell responses

Andrés C.1, Plana M.1, Crespo A.C.1, Alvarez-Fernandez C.1, Climent N.1, Gil C.1, Gallart T.1, León A.2, Clotet B.3, Autran B.4, Chomont N.1, Gatell J.M.1, Sanchez-Palomo S.1, García F.2, DCV2/MANON07-ORVACS Study Group

1Hospital Clinic, IDIBAPS, University of Barcelona, Retrovirology and Viral Immunopathology Laboratory, Barcelona, Spain,
2Hospital Clinic, IDIBAPS, University of Barcelona, Infectious Diseases Department, Barcelona, Spain, 3AIDS Research Institute IrsiCaixa, Institut d’Investigació en Ciències de la Salut Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain, 4INSERM UMR-S 945 - Université Paris VI Pierre et Marie Curie. Hôpital Pitie-Salpêtrière, Paris, France, 5Vaccine and Gene Therapy Institute Florida, Port St Lucie, United States

Background: We recently reported a peak decrease of viral set-point of 1.2 log10 associated with an increase in HIV-1-specific T cell responses in HIV infected individuals receiving autologous myeloid derived dendritic cells (MDDC) pulsed with autologous heat-inactivated whole HIV. Here we assessed if the HIV specific immune responses induced by the vaccine might have cleared some of the reservoir and drove the dynamics of replenishment of viral reservoir during the antiretroviral therapy (cART) interruption.

Methods: We measured total and integrated HIV-1 DNA in isolated CD4 T cells in 36 patients on cART randomized to receive 3 immunizations with MDDC pulsed with autologous HIV-1 (n=24) (DC-HIV-1) or with non-pulsed MDDCs (n=12) (DC-control) at 6 time-points: before any cART, before STOP1 (a first cART interruption 56 weeks before the first immunization to isolate virus for pulsing MDDCs), before and after vaccinations (VAC1 and VAC2) and at weeks 12 and 48 after second interruption of cART.

Results: Vaccinations did not influence HIV-1 DNA levels in vaccinated subjects. After cART interruption post-vaccination (week 12), while total HIV-1 DNA significantly increased in both vaccinees (n=24) and controls (n=12), integrated HIV-1 DNA did not change in vaccinees (1.8 to 1.9, p=0.22) and increased in controls (1.8 to 2.1, p=0.05) (p=0.03 for the difference between groups). HIV-1 specific T cells responses at VAC2 time-point were strongly and inversely correlated with total and integrated HIV-1 DNA after vaccination (r= -0.46, p=0.04 and r= -0.79, p< 0.0001, respectively) and after cART interruption in vaccinees (r= -0.69, p=0.002 and r= -0.82, p< 0.0001, respectively), while a direct correlation was observed in DC-controls (r= 0.72, p=0.03 and r= 0.67, p=0.05 total and integrated HIV-1 DNA after vaccination, respectively) and no correlations were found after cART interruption). These associations were mainly observed with HIV-1 specific T cell responses targeting gag p24 and p17 and nef antigens.

Conclusions: HIV-1 specific T cell immune responses elicited by therapeutic DC vaccines could drive changes in viral reservoir after vaccination and the replenishment of reservoir after cART interruption in chronic HIV-1 infected patients treated at early stages.
Patient-reported receptiveness to a HIV therapeutic vaccine

D. Zucman¹, S. Dimi¹, C. Lalanne², T. Prazuck³, L. Hocqueloux⁴, E. Mortier⁵, I. Auger-Aubin², C. Majerholc¹, O. Chassany², M. Duracinsky²

¹Centre Medico Chirurgical Foch, Suresnes, France, ²Hôpital Saint Louis, Paris, France, ³Centre Hospitalier Régional d’Orléans, Orleans, France, ⁴Centre Hospitalier Régional d’ORLEANS, Orleans, France, ⁵CHU Louis Mourier, Colombes, France

**Background:** There is currently intense research into the development of an HIV therapeutic vaccine. In general, vaccinations are not well accepted by a wider part of the French population. The understanding about acceptance of a therapeutic vaccine in PLWHA remains limited.

**Methods:** Cross-sectional study including HIV-1 infected patients aged 18-75 years in 3 French hospitals. Mixed methods were used. Quantitative study questionnaires: PROQOL-HIV, Brief IPQ-R, a specific questionnaire on vaccination. Qualitative study: semi structured interviews recorded, content analysis of patients’ transcripts with a predefined framework.

**Results:** 220 patients participated, including: 70% men (49% MSM), 10% IDU, 31% migrants. Mean age was 49 years [40-57]. Of 96% of patients taking ART, 92% had undetectable viral load. Socio-demographic characteristics and responses to IPQ-R and PROQOL-HIV were representative for PLWHA in France. Vaccination coverage for hepatitis B, diphtheria, tetanus and polio was high 75%. Some knowledge about a therapeutic vaccine was reported in 54% of patients. 53% of patients considered that acceptable therapeutic vaccine would fully suppress virus. ART interruption for at least 3-6 months would be acceptable for 40% and 44% hoped for definitive ART cessation. Expected benefits were: relief of constraints due to ART in 93% and relief of transmission fears in 84%. Although 30% patients were skeptical about public information on vaccines, 91% would accept a therapeutic vaccine if it was found to be effective and prescribed by their specialist. The willingness to participate to a vaccine clinical trial was 74%. Fear of adverse event was frequently expressed (71%). Most patients (65%) were confident that such a vaccine will be found in the future. Patients having better knowledge about vaccines had better acceptability. Twenty individual interviews (13 men /7 women) were conducted. Patients concerns were largely focused on the route and timing of administration and on potential efficacy. Qualitative data showed the same trends, with great hope for the therapeutic vaccine.

**Conclusions:** This study explored the perception of therapeutic vaccine by a representative sample of PLWHA in France. They are receptive to the idea of receiving a therapeutic vaccine and the confidence in their treating clinician is a key factor of acceptability.
**PE61**

**Vacc-4x/lenalidomide increases naïve CD4 T-cells in well controlled patients on ART with low preART CD4 counts and poor immune reconstitution**

J. van Lunzen\(^1\), G. Pantaleo\(^2\), K. Arastéh\(^3\), G. Fätkenheuer\(^4\), D. Schürmann\(^5\), J. Zeldis\(^6\), D. Jolliffe\(^7\), K. Krogsgaard\(^8\), A. Mørk\(^9\), M.A. Sommerfelt\(^9\)

\(^1\)University Medical Center Hamburg-Eppendorf, Hamburg, Germany, \(^2\)University of Lausanne, Lausanne, Switzerland, \(^3\)EPIMED, Vivantes Auguste-Viktoria-Klinikum, Berlin, Germany, \(^4\)University of Cologne, Cologne, Germany, \(^5\)Chanté-Universitätismedizin, Berlin, Germany, \(^6\)Celgene, Summit, United States, \(^7\)S-Cubed Ltd, Abingdon, United Kingdom, \(^8\)KLIFO, Copenhagen, Denmark, \(^9\)Bionor Pharma ASA, Oslo, Norway

**Background:** A randomized, exploratory, double-blind phase I/II placebo-controlled clinical study was conducted to determine whether the immune modulator, Lenalidomide, in combination with Vacc-4x therapeutic vaccination, could improve CD4 counts in persons with low pre-ART CD4 nadir which often experience incomplete immune reconstitution despite effective ART (Study ID: NCT01704781).

**Methods:** The study was conducted at 3 sites in Germany from 10.2012 - 08.2014 and included study participants with CD4 T-cell counts >200 and < 500 cells/µL on ART. Part A was a Lenalidomide dose escalation study (5mg n=3; 10mg n=3; 25mg n=6) in combination with 4 weekly intradermal (id) Vacc-4x immunizations (1.2mg) using rhuGM-CSF (60µg) as local adjuvant. Part B used 6 id Vacc-4x immunizations and rhuGM-CSF on ART at weeks 1, 2, 3, 4, 12 and 13 in combination with 25mg Lenalidomide tablets (n=12) or placebo (n=12). Lenalidomide/placebo was administered once daily two days before and on the day of each immunization with follow up until week 26. A two-sample 2-sided t-test (Satterthwaite) compared mean change in CD4 counts from baseline (BL) to week 26 between the two groups. A paired T-test compared change in mean CD4 counts at baseline with week 26 within each group.

**Results:** In the part B ITT population (n=24), Vacc-4x/Lenalidomide and Vacc-4x/placebo had mean pre-ART CD4 counts of 141 and 99, and mean BL CD4 counts of 365 and 304 cells/µL respectively. A significant mean CD4 increase of 91 cells/µL (p=0.009) was observed between BL and week 26 in the Vacc-4x/Lenalidomide group (n=12). The Vacc-4x/placebo group had a corresponding mean CD4 increase of 42 cells/µL (p=0.100). CD4 increases compared between the two groups, were not statistically significant (p=0.201). Mean naïve CD4 T-cells increased in Vacc-4x/Lenalidomide and Vacc-4x/placebo groups (48 and 21 cells/µL). Other T-cell phenotypes e.g. Treg remained unchanged. CD4 increases were most pronounced between the last immunization (week 13) and study end (week 26). The Vacc-4x/Lenalidomide combination was well tolerated with only one serious adverse event (abcess) deemed unrelated to treatment.

**Conclusions:** Immune restoration by Lenalidomide in combination with Vacc-4x antigen-specific immune stimulation, warrants further study in populations on ART with low CD4 nadir as well as in HIV “functional cure” strategies.
PE62 LB
A first-in-human phase I/II trial demonstrates the safety and the immunogenicity of a lentiviral based therapeutic HIV vaccine eliciting potent polyfunctional multispecific CD8 and CD4 T cell responses in HIV-infected individuals

H. Toussaint, E. Sarry, A. Bejanariu, S. Agaugué, M. Rodriguez, E. Sabbah-Petrover, C. Bauche
Theravectys, Villejuif, France

Background: One approach to reach the functional cure in HIV infected individuals is the development of T cell immune based strategies able to contain viral replication while preserving CD4+ T cells. We assessed the safety and efficacy of a therapeutic anti-HIV1 primeboost vaccine regimen based on intramuscular injection of two integrative lentiviral vectors (ClinicalTrials.govIdentifier: NCT02054286).

Methods: The randomized, placebo controlled trial enrolled 38 HIV infected individuals on suppressive ART and aimed at comparing the safety, tolerability and immunogenicity of the therapeutic vaccine candidate at 3 incremental doses (5.10^6, 5.10^7 or 5.10^8 TU) versus placebo.

The vaccination regimen consisted of two intramuscular injections 8 weeks apart with nonreplicative and self inactivating lentiviral vectors encoding for immunogenic regions of the HIV GAG, POL and NEF proteins under the regulation of the β2microglobulin human promoter.

Vaccine induced HIV specific T cell in peripheral blood were characterized by intracellular cytokine staining in all participants, placebo included, before and after ART interruption and up to 24 weeks after the first injection.

Results: With the lack of any serious adverse events in all 38 participants and no safety concerns related to the treatment, the clinical data confirmed safety and tolerance of the lentiviral based therapeutic vaccine.

Analysis of the immunological data demonstrated the ability of the vaccine to elicit multispecific and polyfunctional cellular immune responses in vaccinated subjects. The vaccine candidate was highly immunogenic at all doses when compared to the placebo group: i) 93% of the vaccinated subjects showed vaccine specific CD4+ and CD8+ T cell responses compared to 66,6% of the placebo group; ii) a high frequency, from 0.097 to 0.874%, of functional T cells able to produce at least 2 or 3 cytokines among IFNγ, TNFα and IL2 was evidenced; iii) a dose effect was observed when comparing the 3 groups, with greater magnitude with the highest dose; iv) sustainable responses were characterized up to 24 weeks.

Conclusions: This first in human study demonstrates the safety, tolerability and immunogenicity of a lentiviral based therapeutic vaccine regimen.

We are currently evaluating the impact of ART interruption of vaccination on CD4 T cell levels, plasma viral load and viral reservoirs of the induced immune response to optimize the design of the planned Phase II.
Complementary and traditional medicines

PE63

”Hard” versus “Soft” HIV Cure: an Anthropological investigation of the Cultural Meaning of HIV Cure in China

Q. Ma1,2, Z.C. Rich1,2, F. Wu3, F. Hu2, Y. Cheng3, W. Cai2, X. Tang2, J.D. Tucker1

1University of North Carolina at Chapel Hill, University of North Carolina Project-China, Guangzhou, China, 2Guangzhou No. Eight People’s Hospital, Guangzhou, China, 3Sun Yat-sen University, Department of Anthropology, Guangzhou, China

Background: As global HIV cure research accelerates, the meaning of “cure” as a concept expands. People may adapt the concept of cure according to their local cultural context. This research aims to investigate the cultural meaning of HIV cure as articulated by HIV-infected individuals, principal investigators (PIs) on HIV cure research studies, HIV physicians and public health experts in China in order to increase the cultural sensitivity of ongoing HIV cure research and optimize researcher-participant communication.

Methods: From August to December 2014, we conducted in-depth interviews with stakeholders including 36 HIV infected individuals, four PIs, and four public health experts in Guangzhou, China. The stakeholders were selected because of their importance in planning, implementing, and funding HIV cure research studies. The interviews were audio-recorded, transcribed and translated into English for analysis. A code based methodology was used to identify themes and structure the analysis.

Results: We identified a clear dichotomy in the terms used to describe HIV cure research. Our data suggests that medical professionals in China, in line with their western counterparts, adopted the biomedical definition of cure which focuses on viral eradication. We refer to this concept as a “hard” cure. However, the “hard” cure generated confusion among HIV-infected individuals who were unclear about the meaning of this for their individual lives. In contrast, HIV-infected individuals preferred to use a different Chinese term that we called the “soft” cure. The “soft” cure resonates with traditional Chinese medicine philosophy and focuses on restoring yin/yang balance regardless of viral status. This concept clearly denotes improvement in symptoms, but leaves some uncertainty about virological status and assay results. Although “soft” cure seemed more acceptable among HIV-infected individuals, physician researchers felt that this was not sufficiently specific and “hard” cure was more useful.

Conclusions: The “hard” or “soft” cure dichotomy is analogous to the tension in describing ongoing research as “cure” or “remission.” Using a “soft” cure concept may provide a clearer and more culturally sensitive way of discussing HIV cure research with potential research participants and other HIV-infected individuals. This may also help re-align expectations with the early stage of cure research.
Reduction in total HIV-1 proviral DNA following re-boost immunizations using the peptide-based therapeutic vaccine candidate, Vacc-4x, during ART


1Universitätsklinikum Bonn, Medizinische Klinik und Poliklinik I, Bonn, Germany, 2University of California at Davis, Sacramento, United States, 3University of Lausanne, Lausanne, Switzerland, 4Irsicaixa Foundation, Hospital Universitari ‘Germans Trias i Pujol’, UAB, UVIC-UCC, Badalona, Spain, 5Infectious Disease Service Hospital Universitari de Bellvitge, Barcelona, Spain, 6University Medical Center Hamburg-Eppendorf, Hamburg, Germany, 7EPIMED, Vivantes Auguste-Viktoria-Klinikum, Berlin, Germany, 8UCLA Care Center, University of California at Los Angeles, Los Angeles, United States, 9Dept. Infectious Diseases, King’s College London, London, United Kingdom, 10IRCCS San Raffaele, Dept. of Infectious Diseases, Milan, Italy, 11S-Cubed Ltd, Abingdon, United Kingdom, 12Bionor Pharma ASA, Oslo, Norway, 13KLIFO, Copenhagen, Denmark

Background: This study (2012/1 - NCT01712256) investigated the impact of booster immunizations on sustaining vaccine effect in a therapeutic HIV vaccine setting. The effect of two Vacc-4x booster immunizations on total proviral DNA during ART, and on viral load (VL) set-point following a new ART interruption were determined.

Methods: At weeks (w) 0 and 2, eligible study participants from the clinical study (2007/1 - NCT00659789) were given intradermal (i.d.) Vacc-4x booster immunizations (1.2mg) on ART with GM-CSF (60µg) i.d. as a local adjuvant. At w12, ART was interrupted for up to 16 weeks (w28). Study participants were thereafter followed on ART until w36. Total proviral DNA was measured at w0,4,16,28 and 36 using real-time PCR (Taqman) targeting the gag gene. VL set-point was defined as the mean of the last two VL values prior to ART resumption. All study participants provided signed informed consent. The per protocol population (PP) included participants with no major deviations that would challenge the validity of the data.

Results: This open, multicenter, clinical study conducted from 12.2012 to 01.2014 enrolled 33 participants from 9 clinical trial sites within the US and Europe. In the PP, a statistically significant reduction in total proviral DNA (49%) between w0 and w4 was observed (Wilcoxon signed rank p-value 0.030, n=26) which could suggest immune-based killing of infected cells while on ART. The duration of ART prior to the first reboost immunization was mean 36 months (n=22) (min 26; max 47 months). The VL set-point in this study (2012/1) had a geometric mean (GM) value of 26279 copies/ml and was significantly lower than the pre-ART VL set-point (GM VL 74048 copies/ml) (p=0.021, n=13). The VL set-point in this 2012/1 study (GM VL 18162 copies/ml) was reduced compared to the 2007/1 study VL set-point (GM VL 22035 copies/ml), however the difference was not statistically significant, paired t-test p-value 0.453 (n=18).

Conclusions: Vacc-4x booster immunizations safely restored virus control to the VL set-point established following primary Vacc-4x therapeutic vaccination. The reduction in total proviral DNA supports the potential for Vacc-4x therapeutic vaccination to impact on HIV reservoirs during ART and to contribute to HIV cure strategies.
PE65

Optimized antiretroviral therapy during allogeneic hematopoietic stem cell transplantation in HIV-infected individuals

Cash A.1, Capoferri A.1,2, Xu D.1, McHugh H.L.1, Laeyendecker O.1,2, Sakoian S.1, Tony L.1, Bullen C.1, Pohlmeier C.1, Pham P.1,2, Lai J.1, Gallant J.E.6, Siliciano R.F.1,2, Flexner C.1, Pratz K.4, Levis M.4, Jones R.J.4, Kasamon Y.L.4, Ambinder R.F.4, Durand C.M.1,4

1Johns Hopkins University, Department of Medicine, Baltimore, United States, 2Howard Hughes Medical Institute, Baltimore, United States, 3National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, United States, 4Johns Hopkins University, Sidney Kimmel Cancer Center, Baltimore, United States, 5Johns Hopkins University, Department of Pharmacy, Baltimore, United States, 6Southwest CARE Center, Santa Fe, United States

Background: A reservoir of latently infected memory CD4+ T cells is a major barrier to HIV cure. With allogeneic hematopoietic stem cell transplantation (alloHSCT), host hematopoietic cells are replaced with donor hematopoietic cells after cytotoxic therapy and graft versus host (GVH) effects. If antiretroviral therapy (ART) is continued during alloHSCT, it should protect donor hematopoietic cells from infection and result in a reduction or elimination of HIV. However, ART is often interrupted during alloHSCT due to drug interactions, mucositis and vomiting, or organ dysfunction.

Methods: We performed a pilot study on the safety and feasibility of continuing optimized ART during alloHSCT in HIV-infected individuals being treated for hematologic malignancy. Optimized ART included:

1) avoidance of ritonavir-based ART to minimize drug interactions,

2) ART changes for organ dysfunction and

3) subcutaneous enfurvirtide (ENF) during post-transplant cyclophosphamide and if oral ART was not tolerated.

Primary endpoints were incidence of adverse events (AE) from ENF and maintenance of ART through day 60. Secondary outcomes included HIV persistence measures.

Results: Six HIV+ individuals enrolled; five received alloHSCT and one died from malignancy prior to alloHSCT. The remaining 5 patients tolerated ENF without AEs. Patients 1-4 reached day 60 without interruption of ART but required ART changes. Patient 1 achieved 100% donor chimerism by week 8, with undetectable plasma HIV and negative viral outgrowth assay (VOA). The patient died at week 49 with liver failure. Patient 2 has mixed chimerism (87% donor) at week 52 with undetectable plasma HIV, but positive VOA. Patient 3 achieved 100% donor chimerism by week 4 with undetectable plasma HIV but became non-adherent with ART, and at month 5 had viral rebound and meningoencephalitis. Patient 4 has mixed chimerism at week 24 (73% donor) with undetectable plasma HIV but positive VOA.
<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cancer</strong></td>
<td>Hodgkins</td>
<td>Non-Hodgkins</td>
<td>AML</td>
<td>AML</td>
<td>Burkitt lymphoma</td>
<td>Non-Hodgkins</td>
</tr>
<tr>
<td><strong>Phase of Treatment</strong></td>
<td>Chronic</td>
<td>Chronic</td>
<td>Chronic</td>
<td>Chronic</td>
<td>Chronic</td>
<td>Chronic</td>
</tr>
<tr>
<td><strong>Pre alloHSCT CD4 Count</strong></td>
<td>85 cells/ul</td>
<td>231 cells/ul</td>
<td>274 cells/ul</td>
<td>57 cells/ul</td>
<td>260 cells/ul</td>
<td>227 cells/ul</td>
</tr>
<tr>
<td><strong>Pre alloHSCT Viral Load</strong></td>
<td>79 c/ml</td>
<td>&lt;20 c/ml</td>
<td>&lt;20 c/ml</td>
<td>&lt;20 c/ml</td>
<td>&lt;20 c/ml</td>
<td>&lt;20 c/ml</td>
</tr>
<tr>
<td><strong>PBMC Donor Chimerism</strong></td>
<td>100%</td>
<td>87%</td>
<td>100%</td>
<td>73%</td>
<td>N/A</td>
<td>TBD</td>
</tr>
<tr>
<td><strong>CD3+ Donor Chimerism</strong></td>
<td>100%</td>
<td>74%</td>
<td>100%</td>
<td>95%</td>
<td>N/A</td>
<td>TBD</td>
</tr>
<tr>
<td><strong>Number of ART Changes</strong></td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>N/A</td>
<td>TBD</td>
</tr>
<tr>
<td><strong>ART Maintenance</strong></td>
<td>73%</td>
<td>95-100%</td>
<td>Poor</td>
<td>95-100%</td>
<td>N/A</td>
<td>TBD</td>
</tr>
<tr>
<td><strong>Oncology outcomes, survival</strong></td>
<td>Died at week 49, liver failure</td>
<td>Alive, cancer free at week 76</td>
<td>Alive, cancer free at week 45</td>
<td>Alive, cancer free at week 31</td>
<td>Died, prior to BMT</td>
<td>Received BMT on Jan 2nd 2015</td>
</tr>
</tbody>
</table>

**Conclusions:** During alloHSCT, with optimized ART, it is feasible to maintain ART but regimen changes are common due to drug interactions and organ dysfunction. ENF is a well-tolerated alternative to oral ART. Interruption of ART during alloHSCT can cause a severe acute retroviral syndrome. At early time-points, with mixed chimerism, HIV persists but further studies are needed over time.
Novel therapeutic approaches (including gene therapy)

**PE66**

**VAC-3S immunotherapeutic HIV vaccine combined with ART is immunogenic and safe. Phase II initial analysis of the IPROTECT1 multicenter European study**

Katlama C.1, Rockstroh J.K.2, Gatell J.M.3, Ho Tsong Fang R.4, Girard P.M.5, Slama L.6, Simon A.7, Launay O.8, Cotte L.9, Reynes J.10, Gharakhanian S.11

1AP-HP Pitie Salpetriere Hospital & INSERM, Paris, France, 2University of Bonn, Bonn, Germany, 3Hospital Clinic, Barcelona, Spain, 4InnaVirVax, Genopole Evry, France, 5AP-HP St Antoine Hospital, Paris, France, 6AP-HP Tenon Hospital, Paris, France, 7AP-HP Pitie Salpetriere Hospital, Paris, France, 8AP-HP Cochin-Pasteur & INSERM CIC, Paris, France, 9Croix Rousse Hospital, Lyon, France, 10University Hospital, Montpellier, France, 11Cambridge Innovation Center, InnaVirVax, Cambridge, United States

**Background:** VAC-3S is a novel vaccine directed to the highly conserved gp41, 3S motif of HIV-1. Anti-3S antibodies (Abs) block 3S binding to gC1qR, prevents CD4 surface expression of NKp44L, the natural ligand of NKp44 expressed on activated Natural Killer cells. Anti-3S Abs have anti-CD4 apoptotic effects, in vitro. High 3S Abs are associated with low inflammation biomarkers in SHIV-infected macaques. Anti-3S Abs have been shown to be negatively correlated with HIV DNA. We hypothesize that VAC-3S enables re-establishment of CD4/CD8 homeostasis hence can comprise the immunological component of an HIV functional cure approach.

**Methods:** Prospective, randomized, placebo-controlled, double-blind, 3-step study in Europe, assessing immunotherapeutic properties of VAC-3S at 16, 32, 64 mg with 3 IM base immunizations at 4 wk intervals and 3 maintenance boosters in the 16, 32 mg arms. Ninety HIV ART suppressed pts with 200-350 & 350-500 CD4 c/mm3 planned. Endpoints include: anti-3S Abs (ELISA), T lymphocyte activation/differentiation, HIV DNA, inflammatory biomarkers. Planned analysis after 50% inclusions in first 2-steps.

**Results:** In these first two steps, 56 pts (47 male / 9 female), randomized, and completed vaccinations. Pts are 62% Caucasian, 30% African heritage. Median age 46 years (23-59); BMI 23 kg/m2 (16-33), HIV duration 60 months (1-346); baseline CD4 count 365 cells/mm3 (200-596); nadir CD4 167 cells/mm3 (31-410). One serious Adverse Event (AE) prior to vaccination, one viral rebound post-ART non-adherence. One hundred twenty AEs reported after a total 182 vaccinations, were local (erythema, induration, sensitivity, pain), or systemic (headache, myalgia, vertigo). AEs were mild in 53% pts, moderate in 39% pts, severe in 6% pts. Figure 1 shows immunogenicity. Median CD4/CD8 ratios, at baseline, were 0.48 (0.20-1.46) and 0.66 (0.23-2.90) in the low and high CD4 strata, respectively. At the 12-weeks post-vaccination point CD4/CD8 ratio was 0.49 (0.21-1.120) in the low and 0.57 (0.34-2.82) in the high CD4 strata.
Conclusions: VAC-3S is a novel mechanism immunotherapeutic HIV vaccine. Phase II preliminary results, confirms phase I safety, as well as, immunogenicity for all new dose levels assessed. Scheduled long term evaluation includes CD4/CD8 homeostasis, HIV DNA and biomarkers of chronic inflammation.
PE67 LB

VAC-3S, a safe Immunotherapeutic HIV Vaccine decreased total HIV DNA and increased CD4/CD8 ratio: Phase I Final Results

R. Ho Tsong Fang¹, O. Launay¹, C. Rouzioux¹, B. Autran¹, J. Capeau¹, A. Mélard², M. Marcul¹, R. Calin³, H. Bodilis³, J. Crouzet¹, V. Vieillard⁴, P. Debré⁵, S. Gharakhanian⁶, C. Katlama⁷


Background: VAC-3S is an immunotherapy targeting the conserved 3S motif of HIV-1 gp41. On non-infected CD4+ T cells (CD4), anti-3S antibodies prevent the surface expression of NKp44L, ligand of NKp44 expressed on activated Natural Killer cells. Anti-3S antibodies exert in vitro anti-CD4 apoptotic effects and are associated with protection of CD4 and low inflammation/activation biomarkers in SHIV-infected macaques. In patients, natural anti-3S antibodies are associated with slower disease progression.

Methods: IVVAC-3S/P1 was a prospective, randomized, placebo-controlled, double-blind, dose-escalation study, to assess safety and immunogenicity of 3 VAC-3S intramuscular administrations (weeks 0, 4, 8) at 0.1, 1, 10 and 20µg and 1 booster (week 32) in 1 and 10µg arms, in patients receiving ART with CD4>200cells/mm3 and virologically controlled. Analysis included safety, anti-3S antibodies (ELISA), CD4/CD8 ratio, T lymphocyte activation/differentiation, total HIV DNA and inflammation biomarkers. Responders were defined as anti-3S antibodies above 30 arbitrary units (AU) at week 12.

Results: 33 HIV-1-infected patients (29 men) were enrolled. Age, median [range]: 47 years [32;54], CD4: 710cells/mm3 [311;1187], 89 expected AE for 113 vaccinations: 99% of grade 1-2, 1 cephalgia, 1 tenosynovitis grade 3 in 20µg arm, 1 myalgia grade 3, 1 TLF increase grade 4 in placebos, no related SAE nor viral rebound were reported. A dose-response (p=0.003) was shown with 3 responders among 6 patients in 1µg, 3/6 in 10µg and 5/6 in 20µg arms (responders, N=11). Booster injections were immunogenic. Responders showed a transient CD4/CD8 ratio increase of 0.08 [0.01;0.15] at week 24 (p=0.002). Among all patients, a sustained negative correlation was obtained between differences from baseline in log(HIV DNA copies/106 PBMC) and anti-3S titers at weeks 12 (p=0.027), 36 (p=0.107), 60 (p=0.017, see figure) and 84 (p=0.026).

Among responders, these DNA levels decreased at week 60 and week 84 of -0.07 [-0.83;0.23] (p=0.091) and of -0.15 [-0.41;-0.01] (p=0.015) respectively. No group or dose difference was shown in activation, differentiation and inflammation markers. Functions of anti-3S antibodies are under investigation.
**Conclusions:** VAC-3S is a safe and immunogenic HIV immunotherapy at higher tested doses. The induction of anti-3S antibodies was associated with increased CD4/CD8 ratio and decreased total HIV blood reservoir.
PE68 LB

Perspectives on the acceptability of HCRC trials: the challenges for physicians and PLWHIV (ANRS APSEC)

M. Preau1,2, M. Doumergue3, M. Mora2,4,5, C. Goujard6, C. Protière7, L. Meyer8, J.D. Lelièvre9, F. Raffi10, B. Spire2,4,5, O. Lambotte6, M. Suzan Monti2,4,5

1GReSP Lyon 2 University, Bron, France, 2INSERM U912 SESSTIM, Marseille, France, 3GRePS Lyon 2 University, Bron, France, 4Aix Marseille Université, UMR_S912, IRD, Marseille, France, 5ORS PACA, Marseille, France, 6INSERM U1012 Service de Médecine Interne et Immunologie Clinique, Hôpital de Bicêtre, Le Kremlin Bicêtre, France, 7INSERM U912, SESSTIM, Marseille, France, 8Service de Santé publique, AP-HP et INSERM 1018, Centre de recherche en Epidémiologie et Santé des Populations (CESP), Université Paris-Sud, Villejuif, France, 9INSERM U955, Université Paris 12, and Assistance Publique-Hôpitaux de Paris (AP-HP), Groupe Henri-Mondor Albert-Chenevier, Immunologie Clinique, Creteil, France, 10Hotel Dieu, Nantes, France

**Background:** The latest progress in HIV medicine and research has reinforced the belief that HIV infection might be curable. Recently launched the “Towards an HIV cure” initiative to promote multidisciplinary research for a safe, affordable and scalable cure. The main goal of our survey was to collect data based on patients’ and healthcare professionals’ points of view about the HCRC trials and to identify the diverse motivations and barriers which may influence willingness to participate (WTP). Using data from this first phase, we will create guidelines to ensure that recruitment in these upcoming trials is ethical and patient-focused.

**Methods:** The French survey was recently conducted to collect information on the norms and beliefs related to clinical trials, especially trials without direct benefits in the current post-HAART context. Data were collected from 3 PLWH-only focus group meetings PLVIH (n=21) and 3 healthcare professional-only meetings (n=30).

**Results:** Four women and 17 men participated in the PLWH focus group sessions. Thirteen providers, 10 doctors and 7 caregivers participated in the sessions for healthcare professionals.

An analysis of all six meetings identified three primary and often opposing acceptability ‘positions’ or ‘stances’, which developed over the course of each focus group meeting.

The first position was strongly based on participants’ comparison of the trials with the pre-HAART trial era.

The second position concerned the fact that the new strategies could undermine the progress made by previous work, not only from a clinical point of view, but also from the perspective of PLWH behaviours.

The third position reflects general HIV culture which not only accepts the risks of participating in new trials, but embraces innovation and pushes others to continue innovation.

**Conclusions:** The various topics discussed in the focus group meetings highlighted the profound importance of the individual’s personal history with HIV and also regarding a culture of innovation and the patient provider relationship.
Clinical trials and antiretroviral therapy in children and adolescents

PE69 LB
Low but Detectable IFN-γ Responses against Clade-Matched HIV-1 Peptides in Early-Treated Vertically-Infected Children with Long-Term Sustained Viral Suppression

H. Dieumegard1,2, I. Salem Fourati1,2, A. Le Campion1,2, F. Kakkar3,4,5, J. Brophy6,7, L. Samson6,7, M. Hawkes8, S. Read9,10, A. Bitnun9,10, H. Soudeyns1,2,5, EPIC4 Research Team.

1Centre de recherche du CHU Sainte-Justine, Unité d’immunopathologie virale, Montreal, Canada, 2Université de Montréal, Microbiology, Infectiology & Immunology, Montreal, Canada, 3CHU Sainte-Justine, Division of Infectious Diseases, Montreal, Canada, 4CHU Sainte-Justine, Centre maternel et infantile sur le SIDA, Montreal, Canada, 5Université de Montréal, Pediatrics, Montreal, Canada, 6Children’s Hospital of Eastern Ontario, Ottawa, Canada, 7University of Ottawa, Pediatrics, Ottawa, Canada, 8University of Alberta, Pediatrics, Edmonton, Canada, 9Hospital for Sick Children, Infectious Diseases, Toronto, Canada, 10University of Toronto, Pediatrics, Toronto, Canada

Background: Absence of detectable cell-mediated immune responses to HIV-1 is a recurring finding in early-treated HIV-1 vertically-infected children in whom sustained viral suppression is achieved and maintained for an extended period. It has been assumed that levels of viral antigens were too low to trigger or maintain HIV-1-specific immune responses. However, re-emergence of these responses following viral rebound is well documented. Here, IFN-γ responses were measured in peripheral blood mononuclear cells (PBMC) from 4 early-treated vertically-infected children with long-term sustained viral suppression (Bitnun et al., Clin Infect Dis 59: 1012-1019, 2014).

Methods: PBMC were obtained from 4 children who were initiated on combination antiretroviral therapy (cART) within 72 hours of birth and achieved sustained virologic suppression (HIV-1 viral load < 50 copies/mL). At the time of blood sampling, virologic suppression had been maintained for 3.9 to 8.1 years. IFN-γ production in response to HIV-1 clade-matched peptide pools (clade A [23 pools; 122 peptides] and clade C [22 pools; 121 peptides] consensus peptides) were measured using ELISpot. PBMC from HIV-uninfected subjects and a 29 year old HIV clade C-infected adult without sustained viral suppression (140,573 copies/mL) were used as controls. ELISpot positivity was defined according to standard criteria (>50 spot-forming units [SFU] per 106 cells and >2 SD over negative controls).

Results: Low-level HIV-specific IFN-γ responses were detected in all 4 children but not in HIV-uninfected controls. Responses ranged from 0 SFU to 121 SFU/106 PBMC in Case 1, 0 to 98 SFU/106 PBMC in Case 2, 0 to 165 SFU/106 PBMC in Case 3, and 0 to 98 SFU/106 PBMC in Case 4. These responses were substantially lower than clade-matched IFN-γ responses measured in the control subject without long-term viral suppression (0-1858 SFU/106 PBMC) and significantly lower than anti-CD3, CMV-specific and VZV-specific responses.

Conclusions: Low but significant frequencies of cells producing IFN-γ in response to stimulation with HIV-1 clade-matched peptides were detected in early cART-treated children with sustained viral suppression under cART thereafter. These responses may be contributing to long-term control of HIV replication in vertically-infected children, and these dynamics of host-pathogen interaction may qualitatively or quantitatively differ from those observed in HIV-infected adults.
PE70
Results of a community needs assessment and pilot test of a novel HIV cure research training curriculum

K. Dubé1, J. Taylor2, R. Jefferys1, M. Sharp1, S. Wakefield1, J. Handibode6
1UNC-Chapel Hill, School of Medicine, Collaboratory of AIDS Researchers for Eradication (CARE), Chapel Hill, United States, 2Collaboratory of AIDS Researchers for Eradication (CARE) Community Advisory Board (CAB), Palm Spring, United States, 3Treatment Action Group (TAG), New York City, United States, 4Martin Delaney Collaboratory (MDC) National Community Advisory Board (CAB), San Francisco, United States, 5HIV Vaccine Trial Network (HVTN), Seattle, United States, 6AVAC, New York City, United States

Background: HIV ‘cure’ or remission research demands scientific literacy for participants and researchers regarding participation. An international collaboration of advocates, NGOs and researchers has created a curriculum to facilitate understanding and scientific cooperation. Iterative evaluation with intended audiences is being conducted with community groups during curriculum development.

Methods: During the period of August -October 2014, participants attending HIV cure research training sessions completed an online HIV cure research literacy needs assessment. The needs assessment questionnaire fed into the development of a comprehensive HIV cure research literacy curriculum. Individuals who completed the needs assessment questionnaire were invited to participate in pilots of three different curriculum modules. Participants evaluated the content and usefulness of modules, assessed HIV cure research ‘literacy’ levels and sought real-time feedback from participants to develop and refine subsequent training modules.

Results: Of the n = 42 respondents, 40 (95.2%) found the curriculum module very useful and 1 (2.3%) found it somewhat useful, as opposed to not very useful or not at all useful (1 answer missing). Most participants found the module very easy (32/42; 76.2%) or somewhat easy (12/42; 28.6%) to understand, as opposed to not very easy or not at all easy. In-person talks or forums (27/42; 64.2%) were the preferred method of learning, compared with webinars/conference calls (5/42; 11.9%) or self-paced web-based learning programs (8/42; 19.0%). Participants preferred training topics by the community included therapeutic vaccines (31/42; 73.8%), participation in HIV cure research (27/42; 64.2%) and ethical issues (26/42; 61.9% of participants).

Conclusions: The needs assessment of the curriculum effort showed the usefulness of the training and desires to increase community ‘literacy’ around HIV cure research. Based on the results of the pilot tests and needs assessment questionnaire, 15 curriculum modules were developed and scaled-up by scientific and community liaisons. International training program implementation includes PowerPoint teaching sets, pre-/post-test assessments, online and in-person presentations as well as participatory activities. The curriculum can strengthen community capacity to participate in and make decisions around HIV cure research. However, greater efforts to systematically roll out the literacy tool is needed in diverse community populations.
Bringing community to cure

L. Sylla¹, M. Louella², E. Seelbach¹, T. Andrus³

¹Fred Hutchinson Cancer Research Center, defeatHIV Martin Delaney Collaboratory Community Advisory Board, Seattle, United States, ²Fred Hutchinson Cancer Research Center, defeatHIV Martin Delaney Collaboratory, Seattle, United States

Background: Community engagement is essential for successful HIV Cure Research. Individuals with HIV will need to be aware of the potential and risks of HIV cure research in order to make informed decisions about participating in clinical trials. Those with and affected by HIV are needed for advocacy, ethics discussions, and messaging feedback. The defeatHIV Community Advisory Board (dHCAB) is an effective catalyst for community engagement and feedback between HIV cure researchers and the community, as well as for collaboration among different local HIV-related CABs, such as those for CFAR, ACTU, and HVTU. The dHCAB is the local CAB for the defeatHIV Martin Delaney Collaboratory based at Fred Hutchinson Cancer Research Center in Seattle, WA, one of three NIH-supported cure collaboratories.

Methods: The dHCAB has employed multiple strategies for community engagement, including: community forums with HIV cure research leaders; opportunities to meet Timothy Ray Brown; providing multi-cultural food and music at education events; holding community events at different community organizations and venues; visiting agencies to engage them in cure research; tabling at events such as the annual AIDS walk and pride festivals, theater productions, etc; webinars; active use of social media, including Facebook, Twitter and Youtube. Those on the dHCAB have had the opportunity to attend and present at scientific meetings and to provide input to researchers on protocols, informed consent documents, and recruitment.

Results: Over 1000 community members have participated in dHCAB events and we have reached thousands more through outreach. The dHCAB currently consists of 12 members. Members range in age from high school to senior citizens and include women, gay and straight men, individuals in recovery, newly diagnosed individuals and long term survivors, African-Americans, Asians, Latinos, and Native Americans. Programs typically bring in dozens to hundreds of participants. The defeatHIV investigators consider the dHCAB a valuable asset and partner and have taken feedback to heart. We have learned about community concerns and provided meaningful pathways for education and dialogue.

Conclusions: With creativity, perseverance and respect it is possible to engage the community and researchers in meaningful ways that will advance HIV cure research.
PE72
Planning and Community Engagement for HIV Cure Research in Canada, A Collaborative Program Between National Research Teams and Key Populations

R. Reinhard1,2, J. Brophy3,4, H. Soudeyns5,6, É.A. Cohen1,5, K. Fowke7  
1Institut de Recherches Cliniques de Montréal, Montréal, Canada, 2Ontario HIV Treatment Network, Toronto, Canada, 3Children’s Hospital of Eastern Ontario, Ottawa, Canada, 4University of Ottawa, Ottawa, Canada, 5Université de Montréal, Department of Microbiology, Infectiology, and Immunology, Montréal, Canada, 6CHU Sainte-Justine, Montréal, Canada, 7University of Manitoba, Department of Medical Microbiology, Winnipeg, Canada

Background: The Canadian Institutes of Health Research (CIHR), the International AIDS Society (IAS) and the Canadian Foundation for AIDS Research (CANFAR) jointly sponsored team grants to find a cure for HIV. The two funded Canadian teams, respectively, plan to investigate: 1) unique HIV reservoir compartments, notably in myeloid cells, and 2) pediatric reservoirs after early treatment. Cures for HIV may be possible if collaborative research proceeds with community engagement. Much work remains to establish meaningful discussion between researchers and HIV-affected communities. Initiating community engagement discussions in Canada benefits the teams, and the Canadian experience may be relevant to other countries because of the variety of key populations.

Methods: Local networks identified key population participants for community engagement meetings in Montréal (French language, November, 2014), Toronto (January, 2015) and Vancouver (April, 2015). Enhanced privacy rules supported adolescent participation. Community knowledge users and researchers provided facilitation and educational content. Restricted meeting size allowed for discussion to:

1. Raise awareness about cure research;
2. Solicit responses to research questions, including: a) blood/tissue sampling practices, b) cultural, gender-based and other beliefs, and c) sampling recruitment/engagement outside urban centers;
3. Solicit ideas for long-term public communication infrastructure.

Additional feedback was collected via online surveys and written evaluation.

Results: People with HIV, MSM, women, youth, Africans, Caribbeans and Blacks (ACB), hemophiliacs, and Indigenous people participated and rated content and engagement favorably. Certain groups (e.g. HIV/HCV coinfected, IDU, transgender populations) were not yet successfully recruited to participate to the intended degree. Participants reported high levels of comprehension and interest in research; some expressed concern with lack of engagement historically. Meetings produced concrete suggestions for long term communication infrastructure, favored means (e.g integrated social media for youth, regular in person meetings for specific groups), and connection to remote populations or other provinces. Opportunistic sampling was supported as was encouragement to address population dispersion issues typical in Canada.

Conclusions: Communities expressed great interest to support HIV cure research. Challenges remain to engage all groups equitably and address their specific needs. Researcher commitments to sustained and personalized engagement are critical to success and to advance discussion of research questions.
PE73
The transition from incurable to curable: Pediatric leukemia, psychological dimensions of new disease cures, and implications for HIV

C. Gliwa1,2, M.E. Grewe3, R. Necochea1, S. Rennie1, J. Tucker2,3
1University of California Los Angeles, Los Angeles, United States, 2University of North Carolina at Chapel Hill, Chapel Hill, United States, 3UNC Project-China, Guangzhou, China

Background: Although many aspects of HIV cure research are entirely without precedent, the history of medicine offers a rich source for exploring how curative research transforms clinical practice and perceptions of disease over time. This paper examines the history of clinical care for children with acute lymphoblastic leukemia (ALL) as research turned the disease from incurable to curable, and identifies potential implications for pediatric HIV cure research.

Methods: We conducted archival research using primary sources from the M.D. Anderson Cancer Center and reviewed published secondary sources to explore the history of pediatric ALL as it transitioned from an incurable illness in the 1950s to a curable disease by the 1980s. We examined how this transition influenced health systems for clinical management of ALL and perceptions of the disease.

Results: Prior to the 1950s, ALL was a uniformly fatal disease. Children with ALL were not expected to live more than a few months and treatment was largely palliative. However, new chemotherapies developed beginning in the 1940s opened up the possibility of cure, and by the 1980s, half of all patients could be expected to survive five years or longer. With survival now a viable endpoint, psychosocial care for patients had to likewise transition from preparing patients and their families for death to preparing them to deal with an ambiguous future. Dr. Jan van Eys, a pediatrician and bioethicist, claimed in the late 1970s that patients could not truly be called “cured” until their bodies, minds, and lives were healthy. He conceptualized a “tripartite cure”, consisting of biological, psychological, and social elements. This new framework encouraged multi-disciplinary teams to care for children with ALL and develop non-clinical interventions to improve holistic functioning of children.

Conclusions: Children worldwide have enrolled in HIV cure research, but we are likely many years from a durable pediatric HIV cure. The history of ALL and van Eys’ “tripartite cure” remind us that children enrolled in curative trials will have complex psychosocial needs that extend beyond the purview of traditional clinical research. Holistic, multi-disciplinary care will be essential for pediatric disease cure research.
HIV cure goes viral: an analysis of HIV cure #hashtags

K. Muessig1, W. Whipple2, M. Grewe3, A. Gilbertson4, J. Tucker4, A. Thomas5, E. Kelly1, N. Wang6, L. Hightow-Weidman4

1University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Chapel Hill, United States, 2University of North Carolina at Chapel Hill, Center for Bioethics, Chapel Hill, United States, 3University of North Carolina at Chapel Hill, Global Health and Infectious Diseases, Chapel Hill, United States, 4University of North Carolina at Chapel Hill, Medicine, Chapel Hill, United States, 5Proteus Associates/University of Oxford, Oxford Internet Institute, Oxford, United Kingdom, 6University of Oxford, Oxford Internet Institute/Mathematical Institute, Oxford, United Kingdom

Background: Media reports about HIV cure research have powerfully shaped public perceptions of HIV cure. The purpose of this study was to examine the spread of HIV cure research-related Tweets prior to and following the Conference on Retrovirus and Opportunistic Infections (CROI) announcement of the Mississippi child.

Methods: We purchased a data set containing all Tweets (285,581 total Tweets/retweets) with our chosen search terms and utilized a big data analysis program called Splunk® to examine temporal trends. In total, we identified 106,805 HIV cure related Tweets and retweets within the 48.9 days prior to and 74.1 days following the CROI announcement of the Mississippi child on 3 March 2013 (BBC announcement 5:26pm EST). We conducted detailed content analysis on a sample of 1,111 Tweets (613 Tweets retweeted 9 or more times and 505 randomly sampled Tweets with less than 9 retweets, minus 7 non-cure Tweets) and compared trends before and after the CROI announcement.

Results: In the 30 days leading up to the CROI Mississippi child announcement, there were an average of 8.26 tweets/retweets per hour related to HIV cure. During the 30 days following the announcement, this average rate increased to 107.27 tweets/retweets per hour. The three accounts with the most posts included the US CDC and two HIV news aggregator accounts. Most Tweets were from individuals (68.05%) or news organizations (18.27%), including the single most retweeted post from the British Broadcasting Corporation (BBC) (20,047 retweets). 52.12% of Tweets linked to a media article; 38.97% consisted of personal opinion/commentary without linking to news articles. Following the CROI announcement, the amount and proportion of positive and neutral Tweets increased (16.20% to 20.49%; and 42.46% to 53.86%), while negative Tweets decreased (15.64% to 9.23%). Only five Tweets (0.45%) came from self-identified HIV-infected individuals, while 71 Tweets (6.39%) came from healthcare/public health-focused organizations.

Conclusions: The CROI announcement of the Mississippi child substantially increased public social media messages about HIV cure research. Many of these messages were from individuals and quickly diverged from the science. HIV-focused organizations were relatively under-represented.