

Global Antiviral Journal



Final Program and Abstract Book

This program is sponsored by University of Massachusetts Medical School





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Global Antiviral Journal

Aims and Scope

Global Antiviral Journal publishes peer-reviewed original works related to international efforts to advance antiviral discovery and development, including full-length articles and short papers, as well as solicited review articles, conference reports, letters and book reviews. Occasional supplements contain conference abstracts presentations and/or posters from international meetings in the fields of virology and antiviral research. The scope of the journal encompasses chemistry and biological advances in the fundamental and clinical study of antiviral diseases and their treatment. Areas covered include HIV, hepatitis B, hepatitis C and emerging viruses, co-infections, vaccines, animal models, pharmacology, microbicides, alternative therapies, viral dynamics and resistance issues.

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Workshop Objectives

The Scientific Committee has designed the Workshop program to ensure that delegates achieve advancement in the following categories:

- Animal Models and Reservoirs
- Cellular Biology and Reservoirs
- Virology, Immunology and Reservoirs
- Pharmacology, Intensification and Reservoirs
- Clinical Implications of Compartments and Reservoirs
- Primary HIV Infection and Eradication Issues
- New Therapeutic Approaches

Welcome to the Sixth International Workshop on HIV Persistence during Therapy

Dear participants,

On behalf of the Steering Committee, I have the pleasure to welcome you to the 6th edition of the "International Workshop on HIV Persistence, Reservoirs & Eradication Strategies" in Miami, December 3-6, 2013.

This edition is special as it is the first time that the workshop takes place in the USA, and also it represents the 10th anniversary of this event. First launched in December 2003 in Saint Martin (FWI), this meeting has received a growing number of participants from one edition to another, with a constant increase in high quality abstracts that are submitted. These evolutions are confirmed this year, with more than 230 registrants and sessions packed with presentations, either oral or in the form of posters.

As usual, we will go from basic science to clinical results and try to find the essential time for discussion...

I personally want to thank Mario Stevenson and David Margolis for their constant support over the years to make this workshop a success.

I want to thank all the members of the Scientific Committee, speakers and poster presenters for this exciting program.

I want to thank the ANRS and the NIH along with the pharmaceutical companies who support this workshop. Without their commitment, nothing would be possible.

Finally, I want to thank Informed Horizons for their invaluable help.

I wish you fruitful scientific exchanges!

Alain Lafenillad

Alain Lafeuillade, MD On behalf of the Steering Committee

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Carine Van Lint Universite Libre de Bruxelles, Gosselies, Belgium

Mark Wainberg McGill University AIDS Centre, Montreal, Canada

Sixth International Workshop on HIV Persistence during Therapy

Scientific Program

Tuesday December 3, 2013

Tuesday Dec	<u>ember 3, 2013</u>	<u>Abstract</u>
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ORAL ABSTRACTS

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Challenges In HIV Eradication Research

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Recent special cases of apparent HIV cure have generated renewed interest in eradication research. The talk will discuss some of the serious challenges that remain in the search for a general approach to curing the infection. First, there is currently no simple and accurate way to measure the size of the latent reservoir in resting CD4+ T cells, which is likely the major barrier to curing the infection. Second, most of the candidate latency reversing agents for the "shock and kill" approach do not effectively activate HIV gene expression in cells from patients, at least compared to maximum T cell activation. Third, a single round of maximum T cell activation induces HIV production in only a fraction of the cells carrying replication-competent viral genomes. Fourth, even after successful induction of HIV gene expression, infected cells do not die from viral cytopathic effects and are not lysed by cytolytic T lymphocytes (CTL) from most patients on antiretroviral therapy. Fifth, the latent reservoir is dominated by viruses with escape mutations in major CTL epitopes. Sixth, a reduction of over 2 logs in the size of the reservoir will likely be required for any clinically significant delay in rebound following treatment interruption. Seventh, genetic evidence suggest the existence of other stable reservoirs. Overcoming these challenges will require innovation and a concerted effort of investigators in the field.

ABSTRACT 2

Signaling Pathways and Epigenetic Mechanisms Controlling HIV Latency

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BACKGROUND: HAART must be continuously maintained to prevent HIV-1 rebound from latent reservoirs. Factors that are responsible for maintenance of HIV-1 latency were identified by independent genome wide shRNA library screens. The mechanism of action of compounds that reactivate HIV proviruses was studied by comparison to T-cell receptor activation pathways.

METHODS: shRNA library screens were performed in Jurkat T-cells. Cells carrying reactivated proviruses were purified by sorting after sequential passages and the shRNA sequences identified by next-generation sequencing and classified by systems biology tools. Naïve T cells were polarized into Th17, Th1, Th2 and Treg cells, infected with a single round HIV-1 proviral clone and forced into quiescence by cytokine restriction to generate latently infected cell populations.

RESULTS: The screen identified many of the shRNAs associated with epigenetic silencing mechanisms, as well as some novel targets including the estrogen receptor, ESR1. In cells where PRC2 (EZH2) was knocked down, all of the inducible proviruses were found in the H3K27me3 population suggesting that PRC2 complex is required not only for maintenance of latency but also for entry of HIV proviruses into latency. High content screens have shown the need to activate both P-TEFb and reverse epigenetic blocks. Evaluation of the mechanisms of action of the T-cell receptor, cytokines, disulfiram, farnesyl transferase and ESR1 inhibitors highlights how activation of unique pathways can lead to proviral reactivation.

CONCLUSION: The reactivation of latent proviruses requires both P-TEFb and transcription initiation. Reversing any number of rate limiting steps can lead to proviral reactivation but in a limited number of cells. This suggests that there will be many opportunities to identify synergies between different classes of proviral activators and design efficient reactivation strategies.

Role of Lysine Methylation in HIV Latency

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New therapeutic strategies are needed to overcome postintegration latency and "flush out" the viral reservoirs to achieve viral eradication. We have previously shown that the lysine methyltransferase Set7/9 and the demethylase LSD1 regulate HIV transcription by modifying the HIV transactivator Tat. Here, we performed a comprehensive lentiviral shRNA screen of all lysine methyltransferases (KMTs) in J-Lat cells to identify new activators or repressors of HIV transcription/latency. We discovered several new KMTs that negatively or positively regulate reactivation of HIV transcription from latency and will discuss their mechanistic influence on HIV transcription.

ABSTRACT 4

A Novel Pathway of HIV-1 Proviral Latency Controlled by Amino Acid Starvation via HDAC4

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BACKGROUND: Epigenetic factors contribute to silencing integrated proviruses and are nowadays targets of experimental approaches. Among other determinants, class I histone deacetylase inhibitors (HDACi) are well-established targets for pharmacologic modulation of HIV-1 latency, whereas the potential role of class II HDACi is debated. We have recently observed that a member of class II HDACs, i.e. HDAC4, was a key element emerging from gene expression profiling of cells undergoing deprivation of essential amino acids (AA). Indeed, either AA deprivation or inhibition of HDAC4 resulted in the upregulated expression of integrated transgenes, but not of their natural homologue. We therefore investigated whether a similar regulatory pathway could be involved in the regulation of HIV-1 proviral latency.

METHODS: Latently HIV-1 infected U1, ACH-2 and J-LAT cell lines, containing proviral HIV-1 DNA integrated in their genome, were used. ChIP assays were conducted using ACH-2 DNA amplified by real-time PCR using primers specific for the LTR promoter. Quantification of HIV-1 RNA transcripts was carried out by a TaqMan assay. HIV-1 production was measured in cell culture supernatants by RT activity assay or by p24 Gag ELISA.

RESULTS: As for integrated transgenes, deprivation of essential AA led to reactivation of HIV-1 transcription and virion expression in HDAC4 positive ACH-2 cells, but not in HDAC4 negative U1 cells in the presence of comparable cytotoxicity. Similar results were obtained with selective targeting of HDAC4, but not of HDAC6, by either selective pharmacologic inhibitors (MC1568 and MC1575) or by siRNA. We have preliminary extended these observations to the latently-infected J-LAT T lymphocytic cell lines. However, although all J-LAT clones tested were positive for HDAC4 expression (like ACH-2), only some of them exhibited increased HIV-1 transcription and production after incubation with MC1568 or MC1575, suggesting that additional factors influence the capacity of this class II HDAC to contribute to a state of HIV-1 latency.

CONCLUSIONS: Our observations suggest that AA starvation and/or HDAC4 modulation contributes to an intracellular response to the presence of integrated DNA of foreign origin. The potentially synergistic use of class I and II HDACi for reactivating latent proviral reservoirs is reinforced by our study.

Dual Role of the Cellular Cofactor CTIP2 in HIV-1 Latency

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Our previous studies reported that the cellular cofactor CTIP2 (Bcl11b) favors the establishment of HIV-1 post-integration latency in microglial cells, the main reservoirs of virus in the brain. CTIP2 recruits chromatin-modifying enzymes to promote a heterochromatin environment at the HIV-1 transcriptional promoter. This epigenetic control clearly favors establishment of latency, but cannot explain the persistence of the reservoirs, which depends on cellular counter-actions aimed at preventing the environmental stimuli. Interestingly, these events rely on the positive transcription elongation factor b (P-TEFb) (CyclinT1/Cdk9) function.

OBJECTIVES: Our objective was to evaluate the influence of CTIP2 on mechanisms that prevent reactivation of HIV-1 latent proviruses. More specifically, we studied the effect of CTIP2 on P-TEFb function.

METHODS: To this end, we used focused and complementary biological, biochemical, molecular and genome wide approaches.

RESULTS: Purification of the P-TEFb-associated complexes and co-immunoprecipitation experiments demonstrated that CTIP2 interacts with an inactive P-TEFb complex containing HEXIM1 and the 7SK snRNA. CTIP2 associates directly with HEXIM1 and, via the loop 2 of the 7SK snRNA, with P-TEFb.

Kinase assays demonstrated that CTIP2 inhibits the Cdk9 kinase activity of P-TEFb *in vitro* and *in vivo*. In addition, chromatin immunoprecipitation experiments showed that CTIP2 recruits the inactive P-TEFb complex to the HIV-1but also to cellular gene promoters. Finally, comparison of CTIP2-sensititive with P-TEFb-sensitive genes revealed that CTIP2 controls the global P-TEFb function. Data regarding the functional role of the CTIP2/P-TEFb interaction in HIV-1 transcription and latency will be presented, as well as the implication of Tat.

CONCLUSION: Our results suggest that CTIP2 has a double impact on HIV-1 latency. By recruitment of chromatinmodifying enzymes, it favors the establishment of latency; and by inhibiting P-TEFb function, it favors the persistence of latent proviruses. CTIP2 has a direct influence on the provirus by impacting the HIV-1 promoter activity and an indirect influence by impacting the gene expression profile of the latently-infected reservoirs.

ABSTRACT 6

Iws1 Connects LEDGF/p75 and Spt6 to Silence HIV-1 Gene Expression in Latently Infected Cells

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The persistence of a latent reservoir containing replicationcompetent integrated proviruses seriously challenges hopes for HIV-1 eradication. Post-integration transcriptional silencing results from the establishment of repressive chromatin structure at the HIV LTR. However, similar to what is observed in productively infected cells, the majority of latent proviruses are also integrated into actively transcribed genes, a mechanism mediated by the integrase cellular cofactor LEDGF/p75. In order to reconcile these seemingly opposite observations, we explored whether LEDGF/p75 could be involved in the establishment and maintenance of HIV latency. Here we isolated a stable complex containing LEDGF/p75 associated with Spt6 and Iws1, two proteins involved in transcriptional and post-transcriptional control of gene expression and chromatin structure. The formation of the LEDGF/p75:Iws1:Spt6 complex depends on Iws1 bridging LEDGF/p75 to Spt6. We found that depletion of Iws1 promoted HIV expression at a post-integration stage. In

addition, we showed that Iws1 is involved in the establishment and maintenance of latent infection. In latently infected cells, depletion of each of the three subunits of the complex resulted in reactivation of HIV promoter activity. Chromatin immunoprecipitation experiments indicated that Spt6 is required to recruit Iws1 and LEDGF/p75 to the silenced integrated provirus and maintains a repressive nucleosome organization at the HIV promoter. Taken together, our results indicate that a complex containing LEDGF/p75, Iws1 and Spt6 is involved in the post-integration silencing of HIV and participates in the establishment and maintenance of HIV latency.

ABSTRACT 7

The NCOR2-Nurr1-CoREST Transrepression Axis Impairs HIV Reactivation in Latently Infected Microglial Cells

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ABSTRACT: Whereas the incidence of HIV-associated dementia (HAD) has declined due to successful antiretrovirals treatment, prevalence of milder forms of HAD, which include asymptomatic neurocognitive impairment (ANI) and minor neurocognitive disorder (MND) has increased. Both ANI and MND are part of what is known as HIV-associated neurocognitive disorders (HAND). However, the molecular mechanisms explaining regulation of HIV activation in the brain remain ill-defined. The Nurr1/ CoREST transrepression pathway has been recently described as a regulator of glial cells response to brain inflammation by limiting over-reactivation of NF-kB-dependent proinflammatory genes. We report here that, unlike in latentlyinfected T-cells, in latently-infected microglial cells (CHME-5/HIV), HIV is induced by pharmacological inhibitors of the CoREST complex chromatin-modifying enzymes LSD1 and G9a/GLP; these inhibitors also sensitized CHME-5/HIV cells for LPS-mediated HIV reactivation. shRNA-mediated knockdown of Nurr1, LSD1, or CoREST yielded similar results. Chromatin immunoprecipitation analysis followed

by high throughput next generation sequencing revealed that upon microglia treatment with TNFa, the nuclear receptor co-repressor 2 (NCOR2/SMRT), which strongly interacts with Nurr1 to facilitate transrepression, is present at the HIV promoter before activation, then recruited at the earliest time points, and then its presence fluctuates over time. Likewise, Nurr1, CoREST, LSD1, and G9a are recruited to the HIV promoter, changing the epigenetic signature (lower H3K4Me/higher H3K9Me2). The repressor role of these proteins in regulating HIV emergence from latency has been confirmed by unbiased shRNA screens for factors involved in maintaining HIV silenced in latently-infected microglial cells. Our data indicate that the NCOR2-Nurr1-CoREST axis plays a role in preventing HIV over-reactivation in microglial cells, and studying this mechanism in detail may provide therapeutic targets for the treatment of HAND.

ABSTRACT 8

Comparative Analysis of Measures of Viral Reservoirs in HIV-1 Eradication Studies

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The latent reservoir in resting memory CD4+ T cells is a major barrier to HIV-1 eradication. This reservoir was originally defined with a virus outgrowth assay which provides a definitive minimal estimate of number of latently infected cells with replication competent virus. As efforts to target this latent reservoir intensify, there is great current interest in finding simpler ways to measure the reservoir. We will present a comparison of eleven different approaches for quantitating persistent HIV-1 in two cohorts of patients suppressed on HAART using the virus outgrowth assay as a standard for comparison. PCR-based assays for cells containing HIV-1 DNA gave infected cell frequencies at least 2 logs higher than the viral outgrowth assay, even in subjects who started HAART during acute/early infection. The ratio of infected cell frequencies determined by viral outgrowth and PCR-based assays varied dramatically between patients. The dramatic differences in infected cell frequencies and the lack of a precise correlation between culture and PCR-based assays raise the possibility that the successful clearance of latently infected cells may be masked by a larger and variable pool of cells with defective proviruses. In the viral outgrowth assay, <1% of proviruses are induced to release infectious virus after a single round of maximum in vitro activation. The noninduced proviruses are generally considered defective but have not been characterized. Analysis of 213 noninduced proviral clones from treated patients showed 88.3% with identifiable defects but 11.7% with intact genomes and normal long terminal repeat (LTR) function. Using direct sequencing and genome synthesis, we reconstructed full-length, intact noninduced proviral clones and demonstrated growth kinetics comparable to reconstructed induced proviruses from the same patients. Noninduced proviruses have unmethylated promoters and are integrated into active transcription units. Thus, it cannot be excluded that they may become activated in vivo. The identification of replication-competent, noninduced proviruses indicates that the size of the latent reservoir-and, hence, the barrier to cure-may be up to 60-fold greater than previously estimated.

ABSTRACT 9

"Digital" Assays for Quantitative Analysis of Persistent Infection

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Current assays for residual HIV in treated patients provide limited quantitative information about the extent and mechanisms of persistent infection. To develop, test and implement eradication strategies, future assays will need to measure biologically relevant properties, including replication competence, with sensitivity and precision that approach the theoretical limit. "Digital" PCR assays, in which samples are partitioned microscopically, have recently emerged as a practical approach to count individual molecules of HIV nucleic acids. Micro-partitioning is a general strategy that can be applied to offer similar improvements for many other types of assays, as well. This presentation will review the benefits and limitations of droplet digital PCR for analysis of HIV DNA. Droplet assays for HIV RNA will be presented and compared for patients on suppressive therapy initiated in either primary or chronic infection. Micro-wells provide an alternative implementation of digital PCR, as well as infectivity assays, and benefits of this format will be discussed. Finally, the potential for these technologies to converge with sequencing and cytometry will be explored, focusing on near-term opportunities and challenges for the eradication research community

ABSTRACT 10

Assessment and Quantification of Cell Associate Unspliced HIV-1 RNA using Reverse Transcriptase Droplet Digital PCR

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BACKGROUND: Quantification of cell-associated HIV-1 RNA is critical to understanding how HIV-1 latency is established and maintained in a reservoir of infected cells during antiretroviral therapy. Droplet digital polymerase chain reaction (ddPCR) can detect and precisely quantify the target nucleic acids without the need for a calibration curve. Previous studies demonstrated that ddPCR is generally comparable to traditional real time-PCR methods for the quantification of both total HIV-1 DNA and episomal 2-LTR circles. In this study, we developed a reverse transcriptase (RT) ddPCR assay and compared to real time RT-PCR for detection and quantification of unspliced cell-associated HIV-1 RNA in both HIV-uninfected cell lines spiked with HIV-infected cells and clinical samples from patients on antiretroviral therapy (ART) with very low-level viremia.

METHODS: To evaluate the ability of RT-ddPCR to measure cell-associated HIV-1 RNA, serially diluted (2.5 to 108 copies/10 μ l RNA input) in vitro transcribed HIV-1 RNA was quantified by RT-ddPCR and real time RT-PCR. We analyzed 8 replicates of the 100-copy standard to assess the accuracy

and reproducibility of RT-ddPCR. Levels of cell-associated HIV-1 RNA were determined in clinical samples from patients on suppressive ART, and from MT2 cells spiked with the latently-infected cell line ACH2.

RESULTS: RT-ddPCR accurately quantified HIV-1 RNA transcripts; the dynamic range was 10 to 105 copies per 10 µl RNA input (R2 > 0.99), which represented similar sensitivity as real time RT-PCR. The 100-copy input showed that the percentage of positive droplets most closely correlated with the expected copy number. For samples with input RNA of 103 to 105 copies, the copies detected was ~10% lower than expected; for samples with <100 copies of input RNA the calculated copy number was slightly higher than expected (mean 1.9-fold difference). Linearity decreased significantly when greater than 105 copies due to saturation of positive droplets formation. HIV-1 RNA measured by RT-ddPCR and real time RT-PCR in samples from co-culture of serially diluted reactivated ACH2 cells together with MT2 cells lines were highly correlated (R2=0.9668). HIV-1 RNA was detected by RT-ddPCR in 14/20 samples (range, 4-284 copies/million PBMC) and by real time RT-PCR in 13/20 samples (range, 6-479 copies/million PBMC), respectively.

CONCLUSIONS: The RT-ddPCR assay detected and accurately quantified cell-associated HIV-1 RNA with a dynamic range down to 10 copies per reaction. RT-ddPCR demonstrated similar accuracy and sensitivity compared to real-time RT-PCR in the measurement of cell-associated HIV-1 RNA in both cell-lines spiked with HIV-infected cells and in patient samples. RT-ddPCR is a promising tool for the quantification of HIV-1 cell-associated RNA.

ABSTRACT 11

Droplet Digital PCR, the New Tool in HIV Reservoir Quantification?

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BACKGROUND: Digital PCR is a relatively old concept for absolute quantification of DNA using PCR, but recent technological developments allowed its wide use. The current state of art technique for performing digital PCR is based on microdroplet technology. Direct absolute quantification relieves the necessity of standard curves and increases assay accuracy. In addition, the end point PCR set-up allows higher assay flexibility and decreases quantitative bias due to variations in PCR efficiency. In the present work, these theoretical advantages were assessed on the QX100 droplet digital PCR (Bio-rad) on various virological markers to assess the possible use of ddPCR in HIV research.

METHODS: First, ddPCR was compared to a highly sensitive method of real-time PCR based quantification of cellular associated spliced and unspliced HIV RNA. Second, different methods of DNA extractions in combination with ddPCR were compared for quantification of total and episomal HIV DNA. Hereby, the maximal amount of restriction digested DNA was assessed in the ddPCR. Third, a touchdown procedure was optimized for an HIV specific primer probe set with a low melting temperature using touchdown ddPCR.

RESULTS: The comparison of the nested real-time quantitative PCR to ddPCR indicated that ddPCR is at least equally sensitive to qPCR but also that false positive negative control samples may interfere with quantification at the level of single copies.

Episomal 2LTR quantification was compared on ddPCR between total DNA extracted DNA and plasmid purified DNA, revealing a higher accuracy of 2LTR measurements in the total DNA extracts.

Assessment of total DNA load in digital PCR reactions revealed a higher tolerance for inhibition compared to qPCR, but a strong influence of the concentration of restriction digestion mix on ddPCR efficiency. Finally, a touchdown procedure revealed that digital PCR can combine a higher flexibility in assay design while retaining accurate quantitative power compared to qPCR

CONCLUSIONS: We transferred 4 assays used in HIV reservoir research to the ddPCR platform. Although ddPCR has some major advantages for low level quantification in HIV reservoir research, some technical hurdles, including the occurrence of false negative control samples need still be addressed to ameliorate the current technology.

ABSTRACT 12

Subtype Independent Amplification and Sequencing of Low Level Viremia in HIV-1 Infected Patients on Combination Antiretroviral Treatment

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BACKGROUND: In patients with very low viral burden, sequencing is often problematic. However, the management of individuals with low-grade viremia after an initial virological response is an important clinical problem. The aim of this study was to enable subtype independent sequencing of patients with very low levels of plasma virus.

METHODS: Plasma samples from 34 HIV-1 infected patients with known viral load, quantified with COBAS TaqMan HIV-1 Test, version 2.0, were analyzed with an ultracentrifugation step prior to sequencing. PCR and sequencing was performed using a combination of the commercially available ViroSeq* HIV-1 Genotyping System (Abbot) and an in-house nesting protocol. Eight different subtypes were represented.

RESULTS: The median plasma HIV-1 RNA was 119 copies/ ml (<20-966 copies/ml). Amplification of the protease gene (PR) region was obtained in 29 out of 34 specimens (85%). The reverse transcriptase region (RT) was amplified in 24 out of 32 specimens (75%). In total, two samples were negative in both PR and RT (6%). Thirteen patients had HIV-1 RNA below 100 copies/ml, of which seven patients had HIV-1 RNA below 50 copies/ml and two patients had HIV-1 RNA below 20 copies/ml. Sequencing was possible in seven out of eight (88%) for PR and in six out of eight (75%) for RT in subtype B patients. In subtype C, 11 out of 13 (85%) patients for PR and ten out of 12 (83%) for RT was successfully sequenced. Sequencing of PR was possible in all of CRF02_AG and CRF01_AE subtypes. In two of the CRF02_AG and in one of the CRF_AE subtypes, only PR sequencing was possible. One subtype F and one subtype G were included with successful sequencing for RT in both but negative for PR in the subtype F specimen. One patient with unsuccessful sequencing was previously known as subtype A.

CONCLUSION: We present a methodologically easy approach to successful amplification and sequencing of low viral burden in plasma. The assay is subtype independent to a large extent and is clinically important in settings with a variety of HIV-1 subtypes.

ABSTRACT 13

Sensitive HIV-1 RNA Detection in Plasma and Cerebrospinal Fluid (CSF) of Patients Receiving Stable Antiretroviral Therapy

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BACKGROUND: The CNS has been proposed as a 'sanctuary' site where HIV-1 replication may continue during seemingly suppressive ART, reflecting either suboptimal drug levels or reduced drug activity. The aim of this study was to optimize a method for the sensitive quantification of HIV-1 RNA in plasma and CSF in order to study HIV-1 persistence during seemingly suppressive ART across a wide range of HIV-1 clades.

METHODS: Serial dilutions of the second WHO International Standard for HIV-1 RNA were prepared in pooled plasma samples and pooled CSF samples obtained from HIV-negative subjects and tested in ten replicates and three independent experiments by a modified Abbott RealTime HIV-1 assay. The assay was used to measure HIV-1 RNA in plasma samples and paired CSF-plasma samples obtained from patients receiving stable ART and infected with HIV-1 clade A, B, C, D, F, G, CRF_AE, CRF_AG, or complex.

RESULTS: The assay 50% and 95% detection thresholds were 1 and 3 HIV-1 RNA copies/ml, respectively. Assay performance was similar with spiked plasma and CSF, with mean differences relative to the standard of 0.03 and 0.02 log₁₀ copies respectively. Among 104 patients with median 6 years (IQR 3-8) of continuous viral load suppression <50 copies/ml on first-line NNRTI-based ART, 52 (50%) showed detectable HIV-1 RNA in plasma at median levels of 3 copies/ml (range 1, 35). Sensitive HIV-1 RNA detection was obtained in both B (31/60, 51.7%) and non-B (21/44, 47.7%) clades and was not associated with demographic (age, gender, ethnicity, risk group), clinical (duration of HIV diagnosis, nadir and current CD4 count, pre-ART viral load, HIV-1 DNA load) or treatment (NNRTI used, NNRTI plasma concentration) parameters. With 34 paired CSF-plasma samples, 11 CSF samples, all with normal laboratory findings, had detectable HIV-1 RNA at median levels of 40 copies/ml (range 2-2028). Median plasma HIV-1 RNA levels were 20 (range 4-48) and 8 (range TND-45) copies/ml for CSF samples with and without detectable HIV-1 RNA respectively.

CONCLUSIONS: Sensitive HIV-1 RNA detection can be reproducibly applied to both plasma and CSF, and can identify patients with plasma and CSF HIV-1 persistence during seemingly suppressive ART.

ABSTRACT 14

Pyroptosis Drives Both CD4 T-Cell Death And Chronic Inflammation In HIV-Infection: Potential Implications for Maintenance of the Latent HIV Reservoir

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BACKGROUND: The progressive loss of CD4 T cells in HIV-infected individuals is the over-arching cause of AIDS. Apoptosis is the mechanism by which productively infected CD4 T-cells die. In contrast, very little is known about how "bystander" resting CD4 T cells die in lymphoid tissues. These cells are refractory to productive HIV infection yet they account >95% of the CD4 T cell losses occurring in many lymphoid tissues like tonsil and spleen.

METHODS: Human lymphoid aggregated cultures (HLACs) were prepared using tonsil and spleen tissue; lymph nodes from consenting HIV-infected volunteers not on antiretroviral therapy were surgically excised and used in immuno-histological staining studies.

RESULTS: Our finding demonstrate that productive HIV infection in activated CD4 T cells from tonsil and spleen (<5%) promotes silent, caspase-3-mediated apoptosis while abortive infection of nonpermissive resting CD4 T cells (>95%) leads to by caspase-1-mediated pyroptosis, an intensely inflammatory form of programmed cell death. In the pyroptotic death pathway, cytoplasmic contents and proinflammatory cytokines including IL-1, are released into the extracellular space. Surprisingly, lymphoid CD4 T-cells, but not CD8 T cells or B cells in the same tissue, are primed to mount proinflammatory death responses as reflected by high-level expression of pro-IL-1[]. These events combine to create a vicious pathogenic cycle where dying CD4 T-cells release inflammatory signals that attract more cells to become abortively infected and die by pyroptosis causing more inflammation. Cell-to-cell transmission of HIV is obligately required to elicit this pyroptotic death response-cell free virions are ineffective Pyroptosis is efficiently blocked by VX-765, a small-molecule inhibitor of caspase-1 that has been shown to be safe in humans. Analysis of lymph nodes from HIV-infected subjects confirms caspase-1 dependent pyroptotic death of bystander CD4 T cells and release of IL-1.

CONCLUSIONS

1. CD4 T-cell death in HIV-infected lymphoid tissues is principally controlled by caspase-1-mediated pyroptosis, an intensely inflammatory form of programmed cell death.

2. Pyroptosis provides a new and exciting nexus between CD4 T-cell death and inflammation with strong implications for HIV pathogenesis, disease progression and potentially HIV latency.

3. Small-molecule inhibitors of caspase-1 could form a promising new "anti-AIDS" therapy that complements current treatment strategies by altering the detrimental host innate immune response to the virus rather than the virus itself.

ABSTRACT 15

Comparison of Latent HIV-1 Reactivation in Multiple Cell Models and Resting CD4+ T Cells from Aviremic Patients

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Studies to examine control of HIV latency and potential reactivation have been hindered by the small numbers of latently infected cells found in vivo. Major conceptual leaps have been facilitated by the use of latently infected T cell lines and primary cells. However, notable differences exist among cell model systems. Furthermore, screening efforts in specific cell models have identified drug candidates for "anti-latency" therapy, which often fail to reactivate HIV uniformly across models. To begin to understand the biological characteristics that are inherent to each model of HIV latency, we compared the properties of five primary T cell and four J-Lat cell models to those obtained with a standard viral outgrowth assay using patient-derived infected cells. A panel of thirteen stimuli that are known to reactivate HIV by defined mechanisms of action was selected and tested in parallel in all models. Our results indicate that no single in vitro cell model alone is able to capture accurately the ex vivo response characteristics of latently infected T cells from patients. We also conclude that specific model systems are biased in favor or against certain signaling pathways.

In a separate study, we probed the integration site distribution in five different in vitro models of latency. Cells were infected and separated into fractions containing proviruses that were either expressed or silent/inducible, and integration site populations sequenced from each. We compared the locations of 6,252 expressed proviruses to those of 6,184 silent/inducible proviruses with respect to 140 forms of genomic annotation. A regularized logistic regression model linking proviral expression status to genomic features revealed no predictors of latency that performed better than chance, though several genomic features were significantly associated with proviral expression in individual models. Proviruses in the same chromosomal region tended to share the same expressed or silent/inducible status if they were from the same cell culture model, but not if they were from different models. Therefore, the silent/inducible phenotype characteristic of latent viruses appears to be associated with chromosomal position, but the molecular basis is not fully clarified and may differ among in vitro models of latency.

Viral Reservoirs and Anti-Latency Interventions in Nonhuman Primate Models of SIV/SHIV Infection

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Although there is no perfect animal model for HIV infection, and each model has its limitations, the available SIV/SHIVmacaque models may be considered the most relevant ones to demonstrate proof-of-concept of strategies aimed at achieving a functional or sterilizing cure. Several strategies, particularly early and prolonged treatment regimens, sometimes combined with therapeutic immunizations, have had relative success in inducing a functional cure, in which upon withdrawal of antiretrovirals (ARV), virus replication is contained by antiviral immune responses. CD8+ cell depletion experiments have demonstrated a significant role of CD8+ cell-mediated immune responses in this functional cure; antibody-dependent cell-mediated virus inhibition (ACDVI) may also be a significant contributor. In an effort toward eradicating infection by reducing viral reservoirs, we are currently exploring reactivation/induction strategies by combining a histone deacytelase inhibitor (SAHA) and protein kinase C activator (bryostatin). In in vitro pulse experiments on rhesus macaque peripheral blood mononuclear cells, this combination has a short-lasting effect on increased acetylation (6-10 h) but longer effect on increased expression of the CD69 activation marker (>24 h). To test this combination in vivo, we are currently using a previously established RT-SHIV model in which juvenile macaques are started 6 weeks after intravenous RT-SHIV inoculation on an ARV regimen (tenofovir, emtricitabine, efavirenz once daily) known to rapidly reduce plasma viremia. Once plasma virus levels will have reached very low or undetectable levels, we will expose ARV-treated animals to cycles of either SAHA or SAHA+bryostatin, with collection of blood, colon and lymph node biopsies to monitor the viral and immunological effects. The data

of this experiment will be important to demonstrate proofof-concept of this pharmacologic induction regimen, and to provide guidance for further optimization of eradication strategies.

ABSTRACT 17

In vivo Analysis of HIV Persistence and Eradication

J Garcia-Martinez

Despite suppressive antiretroviral therapy (ART), HIV reservoirs persist in most patients. The latent reservoir consists of cells harboring transcriptionally silent provirus and the residual active reservoir is characterized by low level viral RNA production. The goal of eradication therapies is to eliminate these viral reservoirs from the body. However, our knowledge of the systemic nature of HIV reservoirs is incomplete. To provide a comprehensive characterization of the anatomical distribution of persistent HIV, we performed a systemic examination of the latent and residual active HIV reservoirs in bone marrow-liver-thymus (BLT) humanized mice undergoing ART. Our results demonstrate that the latent HIV reservoir is broadly disseminated during ART. Furthermore, each of the tissues examined exhibited low level vRNA production indicating that the residual active HIV reservoir is also systemic in nature. Our data demonstrate that HIV reservoirs are broadly disseminated in vivo and provide a quantitative framework for the in vivo efficacy evaluation of HIV eradication interventions designed to deplete HIV reservoirs.

HIV Latency Drug Discovery: Optimizing Drugs to Induce Latent HIV Expression

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To date only a limited number of mechanisms have been identified which induce HIV expression in vitro in latently infected cell lines, primary cell models and ex vivo cells from HIV infected subjects. In some cases these have been shown to work synergistically, suggesting the potential for increased effects through combination, however, many of these are not ideal with respect to safety and tolerability thus novel approaches to build combinations are needed. This talk will highlight challenges in exploiting the most advanced of these approaches, histone deacetylase inhibitors (HDACi) both as an target for developing novel compounds with enhanced selectivity and the potential for improved tolerability and as a bait to identify novel combination therapies with increased efficacy. Specifically, we will describe attempts to understand HDAC selectivity and develop preclinical models to optimize HDAC efficacy in vitro as well as the use of ultra high throughput screening (uHTS) to identify new mechanisms for inducing latent HIV expression using suboptimal concentrations of the HDACi SAHA (EC10; 250 nM) to sensitize latently infected cell lines to the effects of novel interventions. Proof of principle for this screening approach will be highlighted by the identification and validation of farnesyl transferase inhibitors (FTi's) which exhibit synergy with SAHA and other known induction mechanisms suggesting novel therapies can be identified to enhance efficacy in combination.

ABSTRACT 19

Modeling a Cure for HIV in Nonhuman Primates Using Hematopoietic Stem Cell Gene Therapy Approaches

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BACKGROUND: Following hematopoietic stem cell (HSC) transplantation and withdrawal of combination antiretroviral therapy (cART), three previously HIV⁺ patients remain free of measurable replication-competent virus. Although these results suggest that HSC transplantation represents a promising strategy to induce functional cure, further steps are required to better understand the mechanism and apply this therapy to a greater number of HIV⁺ patients. We are developing a model of cART-suppressed HIV infection in the pigtailed macaque, which we will use to test gene therapy- and allogeneic transplant-based cure strategies.

METHODS: Animals are infected with SHIV-1157ipd3N4 ("SHIV-C"), after which a 3-drug cART regimen is initiated. In transplant experiments, autologous HSCs are engineered to resist infection, either through transgenic expression of the anti-fusion peptide mC46, or through zinc finger nuclease (ZFN)-mediated disruption of the CCR5 genetic locus. Engraftment, persistence, and SHIV response of these autologous stem cells, and stem cell-derived lymphoid and myeloid cells, are measured longitudinally.

RESULTS: SHIV-C infection drives positive selection for mC46-modified cells in peripheral blood and secondary lymphoid tissues, resulting in increased CD4⁺ T-cell counts and an enhanced SHIV-specific immune response. Early CCR5 disruption transplants show sustained engraftment of CCR5-disrupted cells following electroporation with ZFN mRNA. These are, to our knowledge, the first demonstrations of engraftment of electroporated HSCs, and of CCR5-disrupted cells, in a large animal model. In separate studies, we observe that administration of three-drug cART to SHIV-C-infected animals leads to rapid and durable suppression of plasma viremia to <30 copies/mL plasma.
CONCLUSIONS: Our pigtailed macaque model of HIV infection and cART represents a promising platform for modeling functional cure strategies. mC46 protects T-cells from SHIV-dependent depletion by enabling host immune cells to antagonize spreading infection; we predict that transplants with CCR5-disrupted stem cells will mediate comparable protection. We are also interested in using this model to evaluate the curative potential of allogeneic transplant. We will model the clinical applicability of these interventions by introducing them into infected, cART-treated animals. We predict that at a sufficient threshold of protection, positive selection for mC46-expressing or CCR5-disrupted cells will lead to cART-independent control of viremia (functional cure) in our animals.

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ABSTRACT 20

Understanding Lentiviral Persistence *in vivo* Using Nonhuman Primate Models

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A primary obstacle in developing a strategy to cure HIV infection is the early establishment of long-lived viral reservoirs comprised of cells or anatomic sites that retain replication competent virus that persists for years even in the face of apparently suppressive antiretroviral treatment that contribute to the rapid recrudescent viremia when treatment is interrupted. There are major gaps in our understanding of viral reservoir establishment, maintenance, phenotype and tissue compartmentalization that are particularly difficult to study in humans but can be readily studied in nonhuman primates (NHP). These NHP models can help identify the tissue and cellular sources of persistent SIV/HIV, which is one of the seven key scientific priorities for HIV cure research described by the IAS Scientific Working Group on HIV Cure. In this talk, I will discuss new, sensitive tools and techniques to identify cells that harbor SIV vRNA (either productively infected cells or extracellularly trapped virions) as well as SIV vDNA detection in cells within tissues in situ that can provide additional insight into reservoir establishment, tissue compartmentalization and phenotype.

ABSTRACT 21

Does Expression of Vpx by SIV Facilitate Infection of Macrophages and Resting CD4 T cells *in vivo*?

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The viral accessory protein Vpx, expressed by certain strains of simian and human immunodeficiency viruses (SIVs and HIVs), is thought to function by degrading the cellular protein SAMHD-1 which limits intracellular stores of nucleotides. Vpx-mediated degradation is thought to improve the ability of viruses to replicate within myeloid cells. Therefore, we infected Asian macaques and African green monkeys (38 animals total) with viruses that express Vpx, do not express Vpx, or express mutated Vpx and examined which cells were targeted by the virus in vivo. While Vpx expression clearly affected viral dynamics in vivo, with decreased viral loads and decreased infection of memory CD4 T cells in vivo, we found that Vpx seemed to have no effect on the ability of viruses to infect myeloid cells. Moreover, viral DNA was only observed within myeloid cells in lymphoid tissues which were not completely depleted of CD4 T cells. When myeloid cells were found to contain viral DNA, evidence of CD4 T cell phagocytosis in vivo was also evident suggesting that the viral DNA within myeloid cells was actually attributed to phagocytosis of SIVinfected CD4 T cells. These data suggest that Vpx does not facilitate infection of myeloid cells and that myeloid cells are not a significant reservoir of SIV-infected cells in vivo.

ABSTRACT 22

Longitudinal Analysis of Infection Frequencies and Genetic Makeup of Intracellular HIV-1 from Tissue Compartments During Long-Term Suppressive Therapy

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

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

RESULTS: Comparison of the infection frequencies between the 2 time points showed similar (<6-fold difference) infection rates of memory T cell subsets from different tissue compartments for most subjects. In agreement with findings for time point 1, infection frequencies of all T cell subsets were higher in subjects treated during chronic infection than acute infection; time point 2 included transitional-memory T cells which were not examined at time point 1 (6-fold higher infection rate in chronic vs acute; p=0.036). Appoximately 30% of the intracellular HIV sequences appeared to encode replication-incompetent virus. Longitudinal phylogenetic analysis revealed the expansion of some HIV genetic populations and the contraction of others with little evidence of viral evolution. In one subject, a clonal species containing a 380bp deletion was dominant, and increased from 71% to 92% over 6 months in peripheral blood effector memory T cells.

CONCLUSIONS: Our findings suggest the pool of HIVinfected resting memory CD4+ T cells typically does not change dramatically over 6 months in different tissue compartments, reflecting a relatively stable HIV-infection frequency during suppressive therapy with the early initiation of effective therapy resulting in a lower reservoir size. The increase of clonal HIV-1 sequences, especially a large deletion mutant, indicates an expansion of cells with integrated proviral DNA rather than active viral replication.

ABSTRACT 23

Persistent Elevation in HIV viremia during cART with Identical WT Sequences Implies Expansion of a Clonal Source

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INTRODUCTION: The source of persistent viremia during suppressive antiretroviral therapy is unknown. We cared for an individual who developed persistent viremia >50 c/ml after 11 years of suppressive cART. To investigate the origin of this viremia, we carried out detailed quantitative and phylogenetic analysis of plasma HIV populations.

METHODS: The patient was enrolled in an NIH HIV natural history study, and had received cART for over 11 years. He had 2 prior brief cART interruptions (months 72 and 108) but plasma HIV RNA returned to <50 c/ml until he developed viremia of 200-300 c/ml persisting >6 months and oral squamous cell carcinoma (SCC). We analyzed HIV populations using single-genome sequencing (SGS) in plasma obtained prior to cART, during suppressive cART, following rebound and during radiation and chemotherapy.

RESULTS: Pretherapy, (HIV RNA=238,000 c/ml, CD4=22 cells/µl) HIV RNA was genetically diverse with no drug resistance mutations detected by SGS. Treatment interruptions resulted in rebound viremia comprising distinct populations of identical sequences containing wild type (WT) and K103N HIV. Low level viremia (330 c/ml; CD4=164 cells/ µl) emerged after 11 y on cART; SGS revealed both WT and multidrug-resistant HIV. The WT population consisted largely of multiple identical sequences; the resistant population comprised diverse variants encoding K103N+M184V. Switch to TDF+FTC+RTG produced a 10-fold reduction of the drugresistant variants, leaving almost only the WT variants. SCC radiation/chemotherapy reduced peripheral CD4 to 50-70 cells/µl, but viremia persisted (60-90 c/ml) and contained only WT HIV, revealing that the source of the WT sequences was likely a stable reservoir, not ongoing replication. Viremia decayed to <50 c/ml with calculated half-life of 187 d. SCC reoccurred and viremia rebounded, which consisted entirely of identical WT sequences. Subsequent treatment for SCC was unsuccessful; autopsy revealed lymph nodes containing histiocytic infiltrates but no metastatic disease.

CONCLUSIONS: WT and drug sensitive viremia >50 c/ml, consisting of identical sequences, can arise and persist on cART. The emergence of clonal, WT viremia on cART and its insensitivity to cART implies that the source of viremia was an expanded clone of HIV-infected cells perhaps including increased HIV production from that clone.

ABSTRACT 24

Anatomic and Cellular Reservoirs of HIV Infection Before and During HIV Therapy

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The location of important anatomic and cellular reservoirs of HIV infection are not well-defined, especially in the setting of antiretroviral therapy. The largest reservoir is in secondary lymphatic organs, however the role of bone marrow and other organs like kidney and lung have been identified as potentially important. In addition, the CD4 T cell is the primary site of virus production in the untreated patient but little is know about the source of virus (when it is detected) in the suppressed patient. Macrophages may be an important contributor to viral persistence and an important reason why eradication may be difficult with current therapies. In this talk I will review what is known about the anatomic and cellular reservoirs of HIV infection and how antiretroviral therapy may affect this. In addition I will discuss factors that might contribute to the persistence of this reservoir.

ABSTRACT 25

Quantitation of Latently Infected Macrophages in Tissues of Suppressed SIV-infected Macaques that Contribute to the Viral Reservoir

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HIV latency has been well established in resting CD4+ T cells and their longevity provides a llfe-long reservoir in HIV infected individuals on combined antiretroviral therapy (cART). In addition to CD4+T cells, HIV and SIV infect cells of the myeloid lineage, monocytes, macrophages and microglia. However, viral latency in these cells in HIV and SIV infected individuals have not been well studied nor has it been shown that these cells provide a reservoir in HIV or SIV in individuals on cART. Quantitative viral outgrowth assays (QVOA) have been validated by many laboratories for resting CD4 cells in HIV and we have developed a parallel assay for SIV, however, such assays are needed for quantitating monocytes, tissue macrophages and microglia.

We have used a well characterized SIV macaque model in which we have demonstrated infection in tissue macrophages early after infection for these experiments.. Virus has been suppressed in this model to low levels (< 10 copies per ml plasma) with two different cART regimens and we have used this model to develop a QVOA assay for monocytes and tissue macrophages. To ensure that the virus isolated in the assay was from myeloid lineage cells and not from CD4+ T cells a sensitive RT-PCR assay was developed to detect T cell receptor genes as part of the assay. Using this assay, we have quantited monocytes in blood and macrophages in tissues of SIV infected and SIV infected suppressed macaques, to quantitate the number of of monocyte and macrophage infected in unsuppressed macaques which has not been done previously. We have also used the assay to quantitate the number of monocytes and macrophages which harbor virus that can be reactivated in this assay.

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ABSTRACT 26

Experimental CD4 Depletion Prior to SIV Infection in Rhesus Macaques Results in Massive Macrophages and Microglia Infection with Rapid Turnover of Infected Cells

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OBJECTIVES: We previously showed that in rhesus macaques (RM) experimental depletion of CD4⁺ T-cells prior to SIV infection resulted in higher viremia, rapid disease progression, and emergence of CD4-independent SIV-envelopes. Here, we performed a new study of RM CD4⁺ T-cell depletion prior to SIV infection to investigate (i) the sources of the viral burden; (ii) the lifespan of productively infected cells; and (iii) the presence of SIV encephalitis (SIVE).

METHODS: Eight RM were treated with a single administration of rhesus recombinant anti-CD4 depleting antibody (CD4R1) while four remained untreated and served as controls. All twelve RM were infected i.v. with SIVmac₂₅₁ six weeks post-depletion, and started ART at day 52 postinfection. We longitudinally collected blood, lymph node (LN) and rectal biopsies (RB), as well as cerebrospinal fluid (CSF) and brain at necropsy. Immunophenotype was assessed by flow cytometry; life span of infected cells was calculated by modeling viral decay during ART; SIV-RNA+ cells were measured by ISH/IHC.

RESULTS: CD4⁺ T-cell depleted RM showed two logs higher post-peak viral load and rapid disease progression compared to controls. Interestingly, CD4 depleted RM experienced (i) large expansion of circulating, pro-inflammatory (CD14^{dim}CD16⁺) monocytes and higher plasma levels of sCD163 (p<0.001 for both values); (ii) massive SIV infection of macrophages in LN and RB, whit macrophages representing 80% of all SIV-RNA⁺ cells; (iii) aberrant activation of microglia - which express high levels of CD163, HLA-DR, Ki-67 and PCNA – that associates with increased frequency of microglial infection and the establishment of severe SIVE; (iv) life span of productively infected cells higher than control animals (1.3 vs. 0.7 days; p=) but remarkably shorter compared to that estimated for macrophages.

CONCLUSIONS: Depletion of CD4⁺ T-cells prior to SIV infection in RM associates with dramatic changes in disease progression, including higher viral load, expansion of proinflammatory monocytes, massive infection of macrophages and microglia, severe SIVE. The net effect of CD4⁺ T-cell depletion is inability to control SIV replication and shift of the pattern of infected cells to macrophages, microglia, and, potentially, other CD4-low cells.

Distribution and Fine Structure Genetic Analysis of HIV in Gut Associated Lymphoid Tissue (GALT) and Blood after Prolonged Antiretroviral Therapy

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BACKGROUND: HIV has profound effects on GALT, even after prolonged cART, but the role of gut compartment in HIV persistence and as source of ongoing replication remains uncertain. Previous studies on gut have amplified HIV sequences from multiple snip biopsies that were pooled at the time of colonoscopy, a strategy that may introduce bias due to sampling variation. We investigated the distribution of HIV infected cells within gut mucosa by analyzing endoscopyderived material as individual or small pools of biopsies.

METHODS: Patients (n=7) with HIV infection and suppression on cART for 2-10 years were enrolled and samples obtained from blood prior to and on cART, and at time of colonoscopy; small jaws forceps were used to obtain random biopsies (c. 30 snips each) from colon and from ileum. Biopsies were collected as individual snips or in pools of 2-8 snips each. Single genome sequences (SGS) from plasma RNA, PBMC-derived DNA, and gut-derived DNA and RNA were obtained and underwent phylogenetic analysis (MEGA).

RESULTS: Representative biopsy samples were obtained throughout the colon in all patients and from ileum in 4/7 patients. SGS have been completed in 4/7 patients; HIV proviruses were genetically diverse both in individual snips and in pooled groups of snips. Analysis of individual snips yielded HIV proviruses in 7/8 snips, with 1-20 sequences recovered from each snip. No evidence of compartmentalization was present in snip-to-snip comparisons. Groups of identical sequences were present and each group involved proviruses identified in multiple snips and in PBMCs, suggesting that cell expansion was not locally confined. No evidence of genetic divergence was detected in gut compared to pre-therapy HIV plasma, even after >10y of cART. In two populations of identical sequences, proviral DNA sequences from colon matched colon derived RNA and contemporaneous plasmaderived RNA.

CONCLUSIONS: HIV proviruses are extensively and uniformly distributed throughout the GALT without clear evidence of sampling variation. Clonally expanded populations present in gut mucosa were not anatomically restricted, and were not divergent from pre-therapy HIV. Detection of the same identical sequences in gut derived DNA/RNA, and plasma suggests that GALT can be a source of persistent viremia on ART.

ABSTRACT 28

Persistent Expression of HIV-1 p24-Gag in Tissues of Patients on cART

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OBJECTIVES: A current challenge to our ability to eliminate HIV-1 from infected individuals is our lack of a cohesive understanding of anatomical/cellular sites of "latent" and/ or active viral reservoirs and compartments. Therapysuppressed patients harbor latent and defective viral genomes and it is controversial whether viral replication also persists, perhaps in tissue sites or cell populations with reduced drug penetrance. Here we investigated one portion of the viral life cycle occurring within gut-associated lymphoid tissue (GALT) biopsies and in autopsy tissues, the production of cell-associated viral antigens, in individuals on suppressive combination antiretroviral therapy (cART).

METHODS: Quantitative immunohistochemistry (qIHC) was used to investigate p24-Gag antigen production in multiple autopsy-derived tissues (acquired <24hrs post-mortem: bone marrow, brain, kidney, intestine, liver, lung, lymph node, prostate, spleen, thymus and tonsil) collected from 3 wellsuppressed (last plasma viral load (pVL) < 50 - 75 copies/mL (c/mL)) and 3 individuals failing therapy (pVL > 10,000c/ mL). From 9-living donors (pVL ranged from < 50 c/mL to > 5-logs) we investigated GALT biopsies with matched blood from multiple time-points separated by up to 10 years. Tissues from HIV-1 uninfected individuals, irrelevant antibodies, and multiple species antigen specific antibodies were used as controls. PCR of HIV-1 specific single viral templates (*env* and *pol/RT*) were generated and sequenced from plasma nucleic acid preparations.

RESULTS: We detected widespread expression of p24-Gag antigen in tissues during otherwise apparently complete viral suppression in the blood. HIV-1 p24-Gag antigen was detected in all tissues examined and was co-localized with markers of lymphoid (p24⁺:CD4⁺) and myeloid (p24⁺:CD68⁺) cell lineages. PCR and sequencing was achieved in nucleic acid preparations from 9 of 14 plasma samples with pVL < 50c/mL.

CONCLUSIONS: These data demonstrate the unexpectedly widespread presence of viral antigens in tissues of otherwise aviremic individuals and reinforce that plasma viral measurements may not be a sufficient measure of suppression of HIV-1 within tissues *in vivo*. Additional studies are required to optimize assessment and quantification of ongoing viral production. This tissue viral index can then be correlated with measured tissue drug concentrations to determine therapeutic tissue levels of cART drugs to aid in optimizing eradication efforts.

ABSTRACT 29

The Non-human Primate Model for Studies of HIV Eradication

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ABSTRACT: While the availability of a large number of very potent anti-retroviral drugs has dramatically reduced the mortality and morbidity associated with HIV infection, no therapeutic strategy that can eradicate or functionally "cure" the infection is yet available. This inability to cure HIV infection is related to the presence of a persistent reservoir of latently infected cells that is resistant to both conventional anti-retroviral therapy (which targets specific phases of the "productive" virus life cycle) and immune-based interventions (which require expression of viral proteins as target antigens). Several species of non-human primates (NHPs) can be infected with species-specific variants of SIV, and comparative studies of SIV infection of natural (i.e., non-pathogenic) and non-natural (i.e., pathogenic) hosts have provided valuable information on the mechanisms responsible for AIDS pathogenesis. More recently, the SIV/NHP model has also been developed and validated for studies of HIV eradication in the setting of fully suppressive antiretroviral therapy (ART).

In this presentation, I will briefly review the opportunities presented by the various NHP models to conduct studies aimed at developing a functional cure for HIV infection. I will then discuss how a typically different pattern of infected cells (both CD4+ T cell subsets-including the recently described subset of CD4+ "stem cell memory" T cells-and macrophages) during natural and non-natural SIV infection of sooty mangabeys and rhesus macaques may allow us to directly test in vivo the relative contribution of specific infected cell populations to the persistent virus reservoirs in ART-treated SIV-infected NHPs. Finally, I will present and discuss the results of an experiment in which we have developed and used a model of autologous hematopoietic stem cell transplantation (aHSCT) in SIV-infected, ART-treated rhesus macaques to interrogate the mechanisms responsible for the establishment and maintenance of the persistent SIV reservoir. We hope that, ultimately, the results of the presented work may help the design of novel strategies aimed at targeting the major sites of viral persistence in HIV-infected individuals.

The Role of Immune-Based Therapeutics in Curing HIV Infection

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Given the challenge of delivering complex, expensive and potentially harmful antiretroviral therapy on a global level, there is intense interest in the development of short-term, well-tolerated regimens aimed at curing HIV infection. Many mechanisms likely contribute to the failure of current antiretroviral drugs to clear the infection, including persistent inflammation and immune dysfunction. Indeed, a number of host immune biomarkers (including expression of PD-1, LAT-3, TIGIT, Ki67 and activation makers) predict the size of reservoir during effective therapy. Theoretically, chronic inflammation and immune dysfunction might lead to HIV persistence by causing virus production, generating new target cells, enabling infecting of activated and resting target cells, altering the migration patterns of susceptible target cells, increasing the proliferation of infected cells, and preventing normal HIV-specific clearance mechanisms from function. The rapidly evolving data on these issues strongly suggest that a vicious cycle might exist in which HIV persistence causes inflammation that in turn contributes to HIV persistence. Untangling the association between the virus and the host immune environment during therapy might lead to novel interventions aimed at either curing the infection or preventing the development of inflammation-associated endorgan disease.

ABSTRACT 31

The Negative Regulators PD-1, LAG-3 and TIGIT are Associated with HIV Persistence and Incomplete Immune Reconstitution During ART

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OBJECTIVE: The mechanisms for HIV persistence during ART are incompletely known. We hypothesized that expression of negative regulators of T-cell activation will contribute to HIV persistence by continuously and actively promoting HIV latency in infected CD4 T-cells.

METHODS: The levels of expression of PD-1, CTLA-4, LAG-3, TIGIT, TIM-3, BTLA, 2B4 and CD160 were measured by flow cytometry on PBMCs from 48 virally suppressed subjects. The frequency of CD4 T-cells harboring integrated DNA, total HIV DNA, 2-LTR circles and cell associated unspliced (CA-US) HIV RNA was determined by qPCR. The enrichment for integrated HIV DNA was evaluated in sorted memory CD4 T cell subsets expressing some of those negative regulators. More specifically, the impact of PD-1 engagement on HIV latency were evaluated in CD4 T-cells isolated from virally suppressed subjects.

RESULTS: Absolute CD4 T-cell counts were negatively correlated with the expression of PD-1, LAG-3 and TIGIT on CD4 T-cells (p<0.0001, p=0.0002, p=0.005, respectively). Interestingly, the frequency of CD4 T-cells harboring integrated HIV DNA was positively correlated with the expression of these markers (p=0.06, p=0.04 and p=0.002, for PD-1, LAG-3 and TIGIT, respectively). With the exception of TIGIT with 2-LTR circles (p=0.009), no association were found between these markers and total HIV DNA, 2-LTR circles or CA-US HIV RNA. Memory CD4 T-cells expressing high levels of PD-1 or LAG-3 were enriched for HIV integrated DNA when compared to their negative counterparts. Engagement of PD-1 with its ligand PD-L1 inhibited viral production induced by TCR stimulation.

CONCLUSION: We identify PD-1, LAG-3 and TIGIT as markers associated with incomplete CD4 T-cell restoration and HIV persistence in virally suppressed subjects. Our data further demonstrate that PD-1 and LAG-3 identify cells carrying integrated HIV DNA. Collectively, the data suggest that a limited number of specific negative regulators of T-cell activation may actively promote HIV persistence during ART.

ABSTRACT 32

Low Tryptophan 2,3-dioxegenase (TDO) Expression is Associated with a Distinctive Tryptophan Catabolism and Preserved Th17/Treg Balance in HIV Elite Controllers

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BACKGROUND: HIV reservoir size is correlated with timing of ART initiation, CD4 T-cell nadir, T-cell activation and CTL specific responses. HIV elite controllers are characterized by enhanced CTL responses associated with a very low HIV reservoir size compared to ART-treated patients. We previously showed that catabolism of Tryptophan (Trp) into Kynurenine (Kyn) via Indoleamine 2,3-dioxygenase (IDO) was correlated with immune activation in HIV infection. Here, we evaluated the relation between IDO-1, IDO-2 as well as another Trp catabolizing enzyme Tryptophan 2,3-dioxegenase (TDO) expression in HIV-infected patients with different clinical outcomes.

METHODS: Plasma levels of Trp and Kyn were measured by isotope dilution tandem mass spectrometry in ARTnaives (n=96), Successfully Treated patients (ST, n=82), Elite Controllers (EC, n=19), and Healthy Controls (HS, n=51). Levels of IL-6 and microbial translocation marker sCD14 were quantified by ELISA. IDO-1, IDO-2 and TDO mRNA expression was quantified by qPCR. Tregs were characterized as CD4+CD25^{high}CD127^{low}FOXP3^{high} and Th17 cells as CD4+IL-17a+ upon PMA/Ionomycin stimulation. Statistical analyses were assessed using unpaired t-test and Pearson correlation test.

RESULTS: ART-naïve patients had lower Trp and higher immunosuppressive Kyn levels *versus* ST and HS. Accordingly Kyn levels and Kyn/Trp ratio were positively associated with plasma viral loads. IDO-1 but not IDO-2 mRNA expression was higher in ART-naïve patients. In contrast, TDO expression was 100 times lower in EC when compared to all other groups. Interestingly, EC had similar low Trp plasma levels to ARTnaïve patients and differ by the absence of accumulation of Kyn, suggesting a distinctive Trp metabolism. However, EC presented similar Th17/Treg ratio and plasma sCD14 and IL-6 levels to HS.

CONCLUSION: Elite controllers displayed low plasma Trp levels without elevated immunosuppressive Kyn levels, dramatically low TDO expression and preserved Th17/Treg balance. These changes suggest a new avenue for HIV control and provide evidence that novel immunotherapeutic strategies modulating Trp catabolism to reduce immune-metabolic activation in turn contribute to HIV-1 eradication.

ABSTRACT 33

All Bark and No Bite: HIV Exploitation of CTL 'Help' in the Absence of Killing

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BACKGROUND: The ability of HIV-1 to rapidly accumulate mutations provides the virus with an effective means of escaping cytotoxic T lymphocyte (CTL) responses. However, the presence of active antigen-cognizant CTL that fail to impact viral evolution or epitope divergence has been shown in high frequency along with high viral load during progression to AIDS. In this study, we describe how subtle alterations in CTL epitopes expressed by naturally occurring HIV-1 variants are capable of 'baiting' pre-existing CTL to react in such a way to provide a selective advantage for HIV-1.

Methods: Cultured human monocyte derived immature DC (iDC) expressing HIV-1 derived peptide variants were cultured with autologous antigen reactive CTL generated *in vitro* from HIV negative donors. Phenotypic and functional characterization of CTL and DC was determined by various immunoassay methods including ELISPOT, flow cytometry, cytotoxicity assays, and live cell imaging.

RESULTS: The in vitro primed CTL proved to respond with full effector function to cognate antigen, and partially respond to select HIV-1 epitope variants by producing pro-inflammatory factors in the absence of target killing. Importantly, instead of dampening the immune response through CTL elimination of antigen-expressing iDC, a positive CTL-to-DC immune feedback loop occurs whereby iDC expressing antigenic variants differentiate into pro-inflammatory mature DC characteristically capable of attracting and activating naïve as well activated memory CD4+ T helper cells. These CTL-'programmed' DC subsequently respond to the T helper signal CD40L by uniquely sprouting interconnected cellular networks of tunneling nanotube-like structures capable of facilitating intercellular transfer of material including HIV-1. Consequently, these CTL-matured DC exhibit a superior capacity to mediate CD4+ T cell trans-infection.

CONCLUSIONS: This discordant induction of CTL 'helper' activity in the absence of killing likely contributes to the chronic immune activation associated with HIV-1 infection, and can be utilized by HIV-1 to promote viral dissemination and persistence. Our findings highlight the need to address the detrimental potential of eliciting dysfunctional memory CTL responses when designing and implementing anti-HIV-1 vaccine strategies.

ABSTRACT 34

Seminal Cytomegalovirus (CMV) Replication is Associated with Increased CD4+ T-cells Immune Activation and Higher Levels of Proviral HIV DNA Reservoir in Effectively Treated HIV-1-Infected Men who Have Sex with Men (MSM)

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BACKGROUND: Asymptomatic CMV replication is frequent in the genital tract of HIV-infected MSM, and is associated with increased immune activation and HIV disease progression. We hypothesize that CMV-associated immune activation could influence the size of the HIV DNA reservoir during suppressive antiretroviral therapy (ART).

METHODS: Paired blood and seminal samples from 53 ART-treated HIV-infected MSM with undetectable HIV RNA in blood plasma were studied (average CD4+T-cells 634/µl; median time on ART: 3.7 years). Levels of CMV DNA were measured in seminal plasma by real time (RT)-PCR, and total HIV DNA (pol), 2-long terminal repeat (2-LTR) circles, and cell-associated HIV RNA (unspliced and multiple-spliced [ms] encoding for tat/rev) were measured in peripheral blood mononuclear cells (PBMC) by droplet digital (dd)PCR. Integrated (proviral) HIV DNA was calculated by subtracting 2-LTR from total HIV DNA. HIV DNA and RNA were normalized to input CD4+T-cells. Levels of CD4+ and CD8+T-cells immune activation (CD38+HLA-DR+) and proliferation (Ki67+) were determined from frozen PBMC by multicolor flow-cytometry. The associations between levels of proviral HIV DNA, cell-associated HIV RNA, immune activation and proliferation in blood and the presence of CMV replication in semen were investigated using non-parametric statistical tests.

RESULTS: Forty-four % of seminal plasma samples were positive for CMV DNA, and 13% had high-level CMV shedding (>5 log10copies/ml). Presence of detectable seminal CMV was associated with higher levels of proliferating (Ki67+) and activated (CD38+HLA-DR+) CD4+T-cells (P<0.05), and a trend towards higher levels of proliferating CD8+T-cells (P<0.1) in blood. Subjects with detectable seminal CMV had a trend towards higher levels of proviral HIV DNA (2.6 versus 2.1 log10copies/million CD4+T-cells, P=0.11) and significantly higher levels of cell-associated [ms]HIV RNA (P=0.04) compared to those without CMV. Presence of high-levels seminal CMV was associated with higher levels of proviral HIV DNA in blood (3 versus 2.2 log10copies/million CD4+T-cells, P=0.05).

CONCLUSIONS: CMV replication in genital tract of MSM is associated with higher levels of systemic CD4+T-cell immune activation and proliferation and with a greater size and transcriptional activity of the HIV DNA reservoir in PBMCs. Interventions aimed at reducing seminal CMV replication and associated immune activation could be important for HIV curative strategies.

ABSTRACT 35

Is there a Pharmacologic Basis for Persistent HIV Replication?

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Despite remarkable advances in the treatment of HIV, the ability to eradicate the virus from an infected individual still evades us. Sensitive HIV-1 RNA assays have shown viral production persists even in individuals with long-term suppression of plasma viral load (pVL) to < 50 copies/mL, which is the current goal of combination antiretroviral therapy (ART); viral rebound occurs rapidly after discontinuation of ART. Thus, patients must remain on therapy indefinitely. The realization that HIV eradication is impossible with current treatment strategies raises two fundamental questions about the pathogenesis of HIV infection: Where and How does the virus persist in the presence of potent ARVs?

Secondary lymph nodes (LN) and gut associated lymphoid tissue (GALT) are the primary sites of HIV replication and where the latent pool of virus is maintained. HIV replication in these tissues causes inflammation and immune activation, collagen deposition and T cell depletion. These immunopathologies persist after initiation of ART, which raises the possibility that replication may continue in lymphoid tissues (LT) even when pVL is suppressed below the limits of quantitation. LT have been shown to be viral reservoirs in non-human primate studies; HIV RNA has been detected in human rectal samples and HIV DNA detected in GALT tissue of well-suppressed patients; and animal studies have shown low concentrations of some antiretroviral drugs (ARVs) in LT compartments.

These data allow the hypothesis that antiretroviral drug (ARV) concentrations in LT might be insufficient to fully suppress replication in these sites. We prospectively treated 12 HIV-infected persons with ARVs and performed multiple samplings of lymph node (LN), ileum and rectum, as well as peripheral blood, to determine intracellular concentrations of the ARVs in these tissues and to measure levels of persistent HIV. We found the LT concentrations of five of the most frequently used ARVs are much lower than in peripheral blood. These lower concentrations correlated with continued

virus replication measured by the slower decay or increases in the follicular dendritic cell network (FDCn) pool of virions associated with the and viral RNA in productively infected cells. Our findings provide support for the hypothesis that ARV concentrations in LT can be insufficient to fully suppress HIV replication. Factors that we believe may influence LT penetration are the physicochemical properties of the ARVs, and patient specific factors, such as host genetic variability in drug metabolizing enzymes or transporter function and lymph node fibrosis. Strategies that might be used to increase drug concentrations in sanctuary sites include modification of currently utilized drugs, blockade of transporters and enzymes that affect drug metabolism and pharmacokinetics, and local drug administration.

A compelling need exists to develop a pharmacologic basis for antiretroviral therapy that will achieve full suppression of viral replication: to avert the long-term consequences of persistent virus production, and because full suppression is a necessary antecedent to the success of any eradication strategy.

ABSTRACT 36

Current and Future Approaches to Quantifying the Relationship Between Pharmacology and HIV Persistence

<u>A Kashuba</u>

A number of anatomic sites have been proposed as the source of persistent viral production including the gastrointestinal tract, the central nervous system, lymphoid tissue, and the genitourinary system. Humanized mice and nonhuman primates are models for HIV infection, persistence, and eradication, yet limited data exist to evaluate inter-species similarities or differences in extracellular or intracellular drug distribution to these sites. This causes difficulty in applying research findings from one species to another, including extrapolating to humans. Additionally, there is limited information on factors responsible for drug distribution and activity at these sites, including protein binding, drug transporter activity, and (in the case of nucleoside analogue reverse transcriptase inhibitors) intracellular endogenous nucleotide concentrations. This presentation will review what is known about species differences and pharmacologic barriers in antiretroviral biodistribution, and what data could be generated to optimize selection of therapeutics to target persistence. New methods to evaluate the relationship between pharmacology and HIV persistence will be reviewed.

ABSTRACT 37

Inhibitory Slopes Show Minimal Variation Within and Across Mechanistic Classes of HIV-1 Antiretroviral Agents and Are Not Likely to Contribute to Differential Effectiveness of Combination Therapy and Viral Persistence

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BACKGROUND: In addition to pharmacological and physical properties and safety, antiviral potency is a main driver of compound selection for development. Recent reports have implicated the slope of the concentration inhibition curve (Hill coefficient) as a potentially important factor, with different classes of antiretroviral drugs (ARVs) exhibiting different slopes in studies evaluating inhibition of HIV-1 replication *in vitro*, and have suggested that different drug and drugs combinations may be more effective in controlling replication and/or be a factor when considering strategies to diminish persistent replication. Here, we re-examine these findings using a kinetic assay that measures the number of infected cells in culture providing a direct measure of the effects of inhibitors on the spread of HIV-1 infection.

METHODS: The kinetic HIV-spread assay employs either target cell or virus encoded fluorescent reporters to measure the number of infected cells in culture over time. We used this assay to determine potencies and slopes of a collection of structurally diverse inhibitors of HIV integrase (InSTIs), protease (PIs), and reverse transcriptase (NNRTIs) over a large potency range. We evaluated these parameters using viruses with differential susceptibility to each drug class as well as different cell types. RESULTS: In peripheral blood mononuclear cells (PBMCs) and lymphoid-derived cell lines, there was little variability in slopes either within a given ARV class or between different ARV classes. InSTIs and NNRTIs both had slopes of ~1.4, while PIs exhibited slopes of ~2.2. Resistance-conferring mutations had the expected effects on overall inhibitor potency but had no effects on slopes. In transfected HEK293T cells, slopes for PIs were steeper and more dependent on assay parameters than for other ARV drug classes.

CONCLUSIONS: Slopes of concentration versus inhibition curves show little variability either within or between different mechanistic classes of HIV replication inhibitors in PBMCs and lymphoid-derived cell lines. For PIs, slopes generated with transfected HEK293T cells are highly dependent on assay conditions, are significantly different than those observed with PBMCs and lymphoid-derived cell lines, and are not likely to reflect *in vivo* dose response slopes. Consistent with clinical experience, the slopes of dose response curves are not likely to be a significant factor in determining clinical efficacy or contribute to the effectiveness of drug specific combinations.

ABSTRACT 38

Farnesyl-Transferase Inhibitors: Identification and Validation of a Class which Reactivates Hiv Latent Expression and is Synergistic with Other Mechanisms *In vitro*

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To date only a limited number of mechanisms have been identified which induce HIV expression *in vitro* in latently infected cell lines, primary cell models and ex vivo cells from HIV infected subjects. In some cases these have been shown to work synergistically, suggesting the potential for increased effects through combination, however, many of these are not ideal with respect to safety and tolerability thus novel approaches to build combinations are needed. To identify novel mechanisms with the potential for use in combination to induce latent HIV, we conducted an ultra-high throughput screen (uHTS) of 2.9 million compounds in the presence of sub-optimal concentrations of the histone deacetylase inhibitor (HDACi) SAHA (EC10; 250 nM) using a T-cell HIV latency model sensitive to compounds which activate HIV latency in primary T-cells (HDACi's, bromodomain inhibitors and PKC agonists). Confirmed hits were titrated in the presence or absence of SAHA (250 nM) and screened for cytotoxicity as well as NFkB activation to eliminate compounds that could cause T-cell activation.

AMONG ~4,500 CONFIRMED HITS, ~800 FARNESYL-TRANSFERASE INHIBITORS (FTI) FROM THREE DISTINCT MECHANISTIC CLASSES WERE IDENTIFIED: 1) peptidomimetic inhibitors which mimic the CaaX peptide of the substrate , 2) non-peptidomimetic inhibitors that coordinate Zn^{2+} ion at the active site and 3) nonpeptidomimetic inhibitors that bind at the 'exit-groove'. We observed a positive correlation between the potency of FTi's in the HIV latency assay (EC₅₀) and in a farnesyl-transferase enzyme assay (IC₅₀) irrespective of mechanism and knockdown of the FT beta subunit induced of HIV expression in this cell model consistent with on target activity.

Importantly, FTi's and SAHA were synergistic both in cell models and resting T-cells isolated from HIV patients suggesting the potential for increased efficacy in combination and providing proof of concept for identifying synergistic combinations using this novel screening paradigm.

What If a Drug that was Developed to Treat HIV Infection Could Actually Help to Cure It?

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BACKGROUND: No previously drug-naïve patient entered into the phase III registrational trials for the novel HIV integrase inhibitor Dolutegravir (DTG) has developed resistance against this compound. To understand this, we selected for resistance against DTG in tissue culture.

METHODS: Different subtypes of HIV-1 were grown in both MT-2 cells and in PBMCs over protracted periods, with the concentration of DTG being increased from 0.05 nM, i.e. 4 times less than the EC50. After 6 months, a final drug concentration of 50-100nM was achieved, beyond which virus could no longer be grown. Viral DNA was sequenced to reveal the presence of mutations that might be responsible for resistance to DTG, and site-directed mutagenesis was performed to confirm biological relevance. Wild-type and mutated recombinant HIV integrase enzymes were also evaluated in biochemical studies.

RESULTS: The most common integrase resistance mutation to arise in subtype B and recombinant 0A/G viruses was R263K followed by E138K or H51Y. With subtype C viruses, G118R arose first followed by E138K or H51Y. R263K alone conferred \approx 3-fold resistance to DTG and a 30% drop in recombinant integrase strand transfer activity, as well as an approximate 30% loss in viral replicative capacity. R263K together with either E138K or H51Y resulted in \approx 6-fold resistance to DTG as well as a more dramatic \approx 80% decrease in viral replication capacity and a \approx 75% loss in integrase enzymatic activity. No compensatory mutation has emerged over >2 years.

CONCLUSIONS: R263K and E138K or H51Y can combine to augment levels of resistance to DTG yet result in a more severe attenuation of viral replication capacity and integrase activity than R263K alone. These findings are in contrast to all previous reports of drug resistance, whereby secondary mutations may increase resistance but also restore virus replication. Since the viruses that contain both R263K and E138K are at a severe replicative disadvantage, these results explain why primary resistance to DTG has not occurred. We propose that the concept of structured treatment interruptions following use of a DTG-based regimen could be explored as a means of trying to eliminate latent viral reservoirs.

ABSTRACT 40

Suppression of Low-level Transcription from Latently Infected Cells and Inhibition of HIV-1 Reactivation by a Potent Tat Inhibitor

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Despite the immense success of HIV anti-retroviral therapy (ART) to reduce replication to very low levels, it fails to eradicate the virus. HIV persists in latently and productively infected CD4⁺T cells in infected subjects undergoing ART. Current therapies are unable to inhibit reactivation of transcription from latently infected resting cells as well as production from active persistent cellular reservoirs. Thus, novel classes of compounds are needed to eliminate this low level viremia that contributes to persistent and damaging chronic immune activation.

The HIV Tat protein, a potent activator of HIV gene expression, is essential for integrated viral genome expression and represents a potential antiviral target. Tat binds the 5' terminal region of HIV mRNA's stem-bulge-loop structure, the Trans-activation Responsive (TAR) element to activate transcription. We found that didehydro-Cortistatin A (dCA), an analogue of a natural steroidal alkaloid from a marine sponge, inhibits Tat-mediated trans-activation of the integrated provirus by binding specifically to the TAR-binding domain of Tat. dCA reduces Tat mediated transcriptional initiation/elongation from the viral promoter to inhibit HIV-1 replication in acutely and chronically infected cells at subnanomolar concentrations. In several cellular models of HIV latency, dCA reduces residual viral production establishing a state of "super latency", rendering very difficult viral reactivation into a productive infection using activators such as TNFa, PMA, or TSA. A concomitant reduction of RNA polymerase II recruitment to "super latent" promoters is observed. Only a strong activator such as Tat can reactivate the virus temporarily. Furthermore, arrest of dCA treatment does not result in viral rebound, as the promoter is transcriptionally shut-off. As expected, latent cell lines either mutated in TAR or Tat are insensitive to dCA. Most importantly, dCA inhibits HIV reactivation upon homeostatic and antigenic stimulation of CD4⁺T cells isolated from virally suppressed patients undergoing ART.

In sum, dCA is an exciting anti-HIV molecule that could inhibit and persistently abrogate residual HIV production from cellular reservoirs in blood and possibly tissues from virally suppressed subjects. Our experiments provide a proofof-concept for the use of transcriptional suppressors in novel therapeutic strategies with the long-term goal of obtaining a functional cure for HIV.

ABSTRACT 41

The Effect of Maraviroc on HIV Transcription in Resting CD4⁺ T-cells from ART-suppressed HIV-1-infected Patients

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BACKGROUND: In previous studies, we have shown that maraviroc could activate NF B and specific target genes in resting CD4⁺-T-cells cells from viremic and ART-suppressed HIV-1-infected patients. The aim of the present work was to determine the effect of MVC on HIV-1 transcription in resting CD4⁺ T cells in blood.

METHODS: MARAVITRANS (Eudra CT: 2012-003215-66) is a clinical trial of 10 days maraviroc intensification that included 10 HIV-infected adults on suppressive ART. Blood samples were collected at baseline, after 1, 3 and 10 days on MVC and 18 days after MVC withdrawal. Activated and resting CD4⁺-T-cells were separated by magnetic beads coupled to monoclonal antibodies (MACS⁺ Technology) and aliquots of 5 million cells were frozen. Cell associated unspliced (CA-US) HIV-1 RNA was quantified in resting CD4⁺ T-cells by real-time PCR. Significant changes in CA-US RNA were determined using Wilcoxon matched-pairs signed-ranks test.

RESULTS: MVC was well tolerated by all the patients throughout the study. Compared to baseline, 7 of the 10 patients had an increase in NF-□B activation (median fold-change, 1.8, IQR 0.9-3.5), including two patients with D/M tropic virus. CA-US HIV-1 RNA was detected at baseline in resting CD4⁺-T-cells in all the patients (mean: 60.8 copies/10⁶ resting CD4⁺ T-cells; 95% CI: 15.5 to 106.2). An increase in CA-US HIV-1 RNA in resting CD4⁺-T-cells was observed in 8 of the 10 patients, including all the 7 patients with a significant fold change increase in NF-kB activity. Compared to baseline, mean fold change in CA-US RNA was 2.59 (95% CI, 0.59-7.99, p=0.33) at day 10 of maraviroc administration and was

2.96 (95% CI, 0.85-14.83, p=0.037) after 18 days of maraviroc withdrawal. A significant increase in MIP-1 β , but not of RANTES or MIP-1 α , was detected in all the patients (from a median 3,3 pg/mL at baseline to a 78.2 pg/mL at day 10).

CONCLUSION: According to these results, maraviroc can activate NF[]B and the subsequent expression of CA-US HIV-1 RNA in resting CD4⁺-T-cells from suppressed HIV-1-infected patients. Through this pathway, MVC could trigger HIV-1 transcription in resting cells thus accelerating the decay of the HIV-1 cell reservoir.

ABSTRACT 42

HIV Sanctuaries, Latency and Reactivation

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Measurement of reservoir and residual replication via exosomes in plasma: TAR and Nef exosomes (as well as their effects on neural cells).

Transcriptional interference in the sense orientation and how the HIV 3'LTR now initiates HIV transcription, thus confounding all ChIP and RNA data of latently infected cells.

Roles of CDK9, CDK11 and CDK12 in HIV transcription, their levels in resting cells and responses to HDACis, BET bromodomain inhibitors and T cell activation signals. These kinases determine transcription elongation and cotranscriptional processing of nascent viral transcripts, i.e. HIV reactivation.

Movement of CDK9 between active and inactive P-TEFb complexes and visual methods for the release of P-TEFb from the 7SK snRNP as measures of effects of HDAC and BET bromodomain inhibitors on HIV reactivation.

Chromatin modifications and movements and how they perturb the P-TEFb equilibrium.

ABSTRACT 43

Anti-PD-L1 Immunotherapy in ARV-suppressed Rhesus Monkeys

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BACKGROUND: The testing of BMS-936559 presently is underway in antiretroviral-suppressed SIVmac251-infected rhesus macaques. The primary endpoint of this study is to assess safety during repeated I.V. administration of BMS-936559, and any improvement to the function of SIV-specific T cells. Our secondary endpoint is to evaluate perturbations in the latent reservoir and viral recrudescence after cessation of ARV therapy.

METHODS: 13 MHC-defined rhesus macaques were confirmed SIVmac251 positive and ARV treatment was initiated, using a 4 drug ARV regimen, for a minimum of 6 months prior to the administration of BMS-936559. All 13 animals received either BMS-936559 (N=8) or isotype control antibody (N=5). Multiple immunologic and virology analyses were used to evaluate ARV regimen efficacy, BMS-936559 dosing safety, BMS-936559 receptor occupancy. Any perturbations in the SIV reservoir in both tissues and blood will also be examined.

RESULTS: We observed durable virologic suppression of SIVmac251 at or below 50 RNA copies/ml in all animals with the application of our well-tolerated, 4-drug regimen. Significant reductions in inflammation markers were observed post ARV treatment. The repeated dosing of BMS-936559 was also well tolerated in all animals with no noted untoward effects. Receptor occupancy of BMS-936559 was favorable. Perturbations to proviral DNA levels (post-BMS-936559 treatment) and SIV viral RNA levels were observed after ARV release.

CONCLUSIONS: Combined, these data provide proof of concept for the safety and the repeated high-level dosing of BMS-936559 *in vivo*.

ABSTRACT 44

Dual Approach to HIV-1 Cure: Activation of Latency and Restoration of Exhausted Virusspecific T Cell Function

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

METHODS & RESULTS: We have developed multiple assays for identification of compounds that reactivate latent HIV-1. These assays involve an integrated and quiescent HIV-1 LTRreporter present in both primary T cells and immortalized T cell lines. A parallel screening approach was employed. Triage of the hits from these screens has revealed compounds capable of activating latent HIV-1 reporters and virus in multiple contexts.

The ability of immunomodulatory therapies to affect human chronic viral infections also is being investigated with *in vitro* assays, in animal models and in clinical trials. As proof of concept, nivolumab (anti-PD-1; BMS-936558) has been tested in the context of chronic HCV in human subjects. A minority of subjects experienced a significant virologic response. Additionally, BMS-936559 (anti-PD-L1) has been administered to SIV-infected rhesus macaques that were suppressed on a combination of antiretrovirals (ARVs). Repeat dosing with anti-PD-L1 appears to be well tolerated and, in some animals, affects SIV rebound after cessation of ARVs.

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

ABSTRACT 45

Challenges and Strategies Towards Functional Cure: How Low Do You Need To Go

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Since the world learned of the "Berlin Patient" 5 years ago, there has been tremendous excitement both in the scientific and lay community about the potential for an HIV "cure". Much has been learned about where and how HIV persists, and a number of provocative studies have demonstrated the potential for antiretroviral therapy, when used very early after acquisition, to lead to antiretroviral-free control. The "Mississippi Child" adds further evidence that preventing wide-spread establishment of a long-lived reservoir may lead to either long-term virologic control or more permanent HIV-1 remission. However, targeting established reservoirs in existing patients has proven to be more challenging. In addition to latency reversing pharmacologic therapies, modalities are being developed to alter host cell susceptibility to infection and allogeneic stem cell transplantation with CCR5 wild-type cells has been shown to reduce the size of latent HIV reservoirs, and can lead to, at a minimum, transient control of HIV-1 infection after treatment interruption. However, mathematical modeling to predict outcomes of therapeutic strategies aimed at reducing the absolute size of the reservoir suggests that nearly the entire replication competent reservoir in the body needs to be purged in order to enable long-term ART-free remission without modifying

cell infectivity or enhancing immune control. Furthermore, antiretroviral treatment interruptions may need to be continued for months to years in order to fully understand the impact of a therapeutic strategy on HIV-1 persistence.

ABSTRACT 46

Virologic and Immunologic Characterization of HIV Reservoirs in Children Following Early Therapy

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We conducted a longitudinal assessment of HIV-1 persistence under long-term suppression of viral replication following early effective combination ART (cART) from infancy. Four youth (median age 16.9 yrs) treated between 0.5 and 2 months of age (early-treated, ET) were studied. Peripheral blood mononuclear cell (PBMC) proviral load and replication competent virus were quantified along with plasma viremia, HIV-1 specific immune responses, and T cell activation markers. Similar analyses were performed on four perinatally HIV-infected youth who began cART in late childhood (latetreated, LT). PBMC proviral load was significantly lower in ET (median 7 copies; range 4-12) than in LT youth (median 181 copies/million PBMCs; range 68-345; p=0.03). Plasma viremia was undetectable in all ET youth whereas all LT had detectable viremia (median 8 copies/ml; range 4-14 copies/ ml; p=0.03). Replication-competent virus was recovered from all LT youth but just one ET youth. HIV-1-specific antibodies were detected in one ET youth. HIV-1-specific T cell responses were not detected in any ET youth. All four LT youth had detectable HIV-specific antibodies (p=0.14) and CD8+ T cell responses (p=0.03). A steady decrease in proviral DNA levels was noted over time in ET youth. We conclude that early effective cART from infancy significantly limits HIV-1 reservoirs and observed continuous decay of proviral reservoirs under long-term control of HIV-1 replication. Continued cART with reduction in HIV-1 reservoirs over time may facilitate HIV-1 eradication or cure strategies.

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ABSTRACT 47

Persistence of HIV-1 Transcription in Patients Initiating Antiretroviral Therapy during Primary Infection

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BACKGROUND: Initiation of antiretroviral therapy (ART) during primary HIV-1 infection (PHI) has been proposed to limit the formation of viral reservoirs. However, it remains unclear whether cells actively transcribing HIV RNA persist in patients treated during PHI, as they do in patients treated during chronic HIV infection. Schmid et al. (*PLoS ONE* 2010) recently reported a profound depletion of HIV-1 transcription in patients who initiated ART during PHI.

We conducted a longitudinal analysis of cell-associated HIV-1 unspliced RNA (usRNA) and total cell-associated viral DNA (vDNA) levels in 33 patients who initiated ART during PHI and were treated for 24 (n=13) or 60 weeks (n=20).

METHODS: Cell-associated HIV nucleic acids were quantified in PBMC at baseline and at 12, 24, 36, 48, and 60 weeks post-ART initiation using seminested real-time PCR.

RESULTS: 71% of patients were at Fiebig stages I-IV at diagnosis, and treatment started at a median of four weeks after the diagnosis. Patients were treated with a triple-class regimen containing a nucleoside backbone, efavirenz, and lopinavir/ritonavir. At baseline, median plasma viremia was 5.38 (IQR: 4.37-5.79) \log_{10} copies/ml and median CD4⁺ T cell count was 535 (325-733) cells/mm³. Plasma viremia dropped to undetectable levels (<50 copies/ml) in 42%, 88%, and 100% of patients at weeks 12, 24, and 60, respectively. From the periods with undetectable plasma viremia on ART, usRNA was detectable in 88%, and vDNA was detectable in 84% of patients. At weeks 12, 24, and 60, respectively, levels of vDNA

decreased from baseline by a median of 0.44, 0.78, and 0.89 \log_{10} , levels of usRNA decreased by 1.34, 1.50, and 1.32 \log_{10} , and plasma viremia decreased by 2.95, 3.68, and 3.73 \log_{10} .

CONCLUSIONS: Cell-associated HIV-1 RNA was detectable in the vast majority of patients who initiated ART during PHI, strongly suggesting the persistence of the active viral reservoir despite early ART initiation. The large disproportion between the relative decreases of cell-free and cell-associated viral RNA from baseline upon ART initiation can be explained by the existence of a class of cells that transcribe viral RNA without viral particle production.

ABSTRACT 48

Small Peripheral Blood HIV-1 Reservoir after Allogeneic (Cord Blood) Stem Cell Transplantation

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BACKGROUND: The Berlin patient is the first patient with a cured HIV-1 infection after allogeneic stem cell transplantation (SCT) with non-functional CCR5 co-receptor (homozygous CCR5 Δ 32) stem cells. Use of homozygous CCR5 Δ 32 cord blood (CB) for SCT allows less stringent matching and as such could be more frequently applied in HIV-infected patients with high-risk hematological disorders. The combination with an HLA-mismatched third party donor (dual-SCT) provides a reduced period of severe neutropenia compared to single CB and may reduce the risk of early infections.

To guide these approaches the UTRECHT (United Transplant Researchers Evaluating a Cure for HIV via Transplantation) platform has been founded.

METHODS: The impact of SCT on the size of the HIV-1 reservoirs was investigated in four patients receiving cART; two dual-SCT (pt1: homozygous CCR5 Δ 32 donor, pt2: CCR5 Wild type (WT) donor) and two standard myeloblative SCT from HLA-identical siblings (pt3: heterozygous CCR5 Δ 32

donor, pt4: CCR5 WT donor). In depth analysis of the HIV-1 reservoir was performed using droplet digital PCR and heminested qPCR (2-LTR circles/proviral DNA cp/million PBMC). Co-receptor usage was determined genotypically.

RESULTS: Two patients who underwent dual-SCT had CCR5 WT alleles and were infected with CCR5-tropic virus (FPR 89.3%; 15.0%). Plasma viral RNA became undetectable (no target detected) after two weeks in patient 1 and five months in patient 2. Proviral DNA levels declined from 324 (pt1) and 18 (pt2) copies (prior to SCT) to 4 copies (2-3 months post-SCT). Unfortunately, patient 1 deceased two months post-SCT after severe pneumonia and relapse of the underlying malignancy. Patient 2 experienced episodes of bacterial sepsis and CMV-reactivation and is 13 months after SCT in remission harboring <8 copies proviral DNA. Patient 3 (heterozygous CCR5 Δ 32) and patient 4 (CCR5 WT) underwent standard allogeneic SCT. Patient 3 harbored 4 copies proviral DNA (5, 11 months post-SCT) and patient 4 harbored <5 copies (2 months post-SCT).

CONCLUSION/DISCUSSION: All our patients demonstrated a small HIV-1 reservoir post-SCT especially as compared to described cases after autologous transplantations. A more rapid decay of viral RNA and DNA was seen after dual-SCT with homozygous CCR5 Δ 32 CB as compared to dual-SCT with CCR5 WT CB.

ABSTRACT 49

Mathematical Model of Spontaneous HIV Infection Control Following Termination of Antiretroviral Therapy

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BACKGROUND: The ANRS VISCONTI study showed that, in a subset of patients, antiretroviral therapy (ART) initiated during primary infection may induce post-treatment control (PTC), i.e., HIV RNA < 50 copies/ml, after treatment termination. Patients that exhibit PTC are not elite controllers (ECs), since they generally lack the clinical traits that have

come to define EC. Our investigation explored the hypothesis that ART initiated during primary infection permits PTC by limiting the size of the latent reservoir, which if small enough at treatment termination, may allow the adaptive immune response to prevent viral rebound and control infection.

METHODS: We modeled within-host HIV dynamics using a mathematical model (differential equations) to capture interactions between target cells, HIV-infected cells, latently infected cells, virus, and cytotoxic T lymphocytes (CTLs). We parameterized the model using published estimates, where possible. For parameters with no existing estimate we assumed a baseline value and performed sensitivity analyses. Results were obtained using linear stability analysis and direct numerical simulations of our model.

RESULTS: Analysis of our model reveals a range in CTL response strengths where a patient may either show viral rebound or PTC (bistability). Our model predicts that in this regime of bistability, there is a threshold in latent reservoir sizes at treatment termination below which PTC can be achieved. Using data on latent reservoir sizes in patients treated during primary infection, our model also predicts viral rebound times for non-controllers.

CONCLUSION: Antiretroviral therapy initiated during primary therapy has been shown to induce PTC in some patients. We have shown that controlling the latent reservoir size is a plausible mechanism through which one may be able to increase the likelihood that control is attained.

ABSTRACT 50

Sigmoid Lamina Propria CD4 T cell Depletion during Acute HIV Infection is Associated with Activated CD4/CD8 T cells, Inflammatory Biomarkers and Viral Burden in the Gut and Blood

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BACKGROUND: Lamina propria (LP) CD4 T cells are severely depleted early in HIV infection, setting the stage for ongoing immune activation and CD4 depletion in chronic infection. Factors contributing to LP CD4 depletion are not well understood.

METHODS: 49 Thais underwent sigmoid colon biopsy: 34 untreated acute HIV-infected (AHI; 12 Fiebig I (FI), 4 FII, 18 FIII and 4 FIV/V), 5 untreated chronically HIV infected (CHI) and 10 HIV uninfected (HU). Immunohistochemistry and quantitative image analysis was performed to measure the CD4 T cell population size within the lamina propria (percent area of LP staining for CD4 T cells). Peripheral blood mononuclear (PBMC) and mucosal (MMC) cells were analyzed by flow cytometry. AHI subjects had sigmoid HIV RNA, plasma sCD14, CRP, D-Dimer, hyaluronic acid, lipopolysaccharide, and cytokines performed. Differences in LP CD4 between groups were compared by Mann Whitney U tests. Spearman correlation was used to assess relationships between CD4% and others variables.

RESULTS: PB CD4 were 437 and 515 cells/mm³ in AHI and CHI respectively. The percent area of the sigmoid LP staining for CD4 T cells (CD4%) in FI (1.27%) was comparable to FII (0.92%, p=0.33) and HU (1.96%, p=0.21), but higher than in

later AHI stages (FIII 0.31%, p=0.003, FIV/V 0.15%, p=0.02). CHI had lower LP CD4 (0.67%) compared to HU (p=0.002), but not statistically different to FI/II (0.96%, p=0.14) and FIII to V (0.27% p=0.17).

For all groups combined, significant inverse correlations were seen between LP CD4% and MMC markers: %CD4+HLA-DR+38+ (-0.37, p=0.006), %CD4+Ki67+ (-0.41, p=0.003), %CD8+HLA-DR+38+ (-0.64, p<0.001) and %CD8+Ki67+ (-0.64, p<0.001). Similar significant correlations were observed with PBMCs: %CD4+HLA-DR+38+ (-0.31, p=0.02), %CD4+Ki67+ (-0.58, p<0.001), %CD8+HLA-DR+38+ (-0.40, p=0.003) and %CD8+Ki67+ (-0.68, p<0.001).

Additionally, in AHI, inverse correlations were seen between LP CD4% and sigmoid HIV RNA (-0.49, p=0.003), blood HIV RNA (-0.36, p=0.02), D-dimer (-0.43, p=0.01), CRP (-052, p=0.002), TNFRII (-0.57, p=0.002), IP10 (-0.43, p=0.02) and neopterin (-0.40, p=0.04).

CONCLUSION: Significant depletion of the sigmoid LP CD4 T cells occurs after Fiebig II AHI and is associated with plasma inflammatory biomarkers, and T cell activation and HIV viremia in the gut and blood.

ABSTRACT 51

Translational Challenges in Targeting Latent HIV Infection

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BACKGROUND: Practical therapies that eradicate HIV infection in patients across the world require safe and reliable approaches to disrupt viral latency, and clear persistently infected cells within a number of months. A single dose of

the potent Class I histone deacetylase inhibitor (HDACi), Vorinostat (VOR) upregulates HIV RNA expression within the resting CD4+ T cells of ART-treated, aviremic HIV+ patients in vivo. But the ability of VOR to repeatedly disrupt latency is unproven, the optimal dosing schema is unknown, and the effect of VOR on host mechanisms that might clear infected cells is uncertain.

METHODS: In a Phase I-II single-center study, HIV+ participants maintained suppressive ART, and resting CD4+ T cells were obtained via leukapheresis. If an increase in resting CD4+ T cell-associated HIV RNA (RC-RNA) was measured following a single VOR 400 mg dose, patients received VOR 400 mg daily M-W for 4 weekly cycles, followed after a 4 week rest period by another 4 weekly cycles. Sparse VOR PK, biomarker measurements of histone acetylation within PBMCs, HIV RNA single-copy assays, RC-RNA, total cellular HIV DNA, and quantitative viral outgrowth assays (QVOA) from resting CD4+ T cells were obtained.

RESULTS: In 5 patients VOR was well tolerated with no adverse events greater than Grade I; mild declines in platelet counts < Grade I were seen commonly. VOR exposures were within expected parameters. However, when measured after dose 11 (2nd dose of cycle 4) and dose 22 (2nd dose of cycle 8) cellular histone acetylation was little increased from baseline levels, and measures of RC-RNA only modestly increased in some patients. QVOA and other assays were also generally stable.

CONCLUSIONS: A complex, multiphasic cascade of host gene expression is triggered following HDACi exposure. Our findings suggest that after HIV latency is disrupted by an initial VOR dose, a time limited refractory period of uncertain duration ensues, prior to the resetting of responsiveness of the viral promoter to HDACi induction. The kinetic nature of these effects may differ between specific HDACis. The implications of these findings for combination strategies to disrupt and clear latent HIV infection will be discussed.

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ABSTRACT 52

Activating Latent HIV with Vorinostat: the Knowns and Unknowns

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Histone deacetylase inhibitors (HDACi) activate HIV virus production in latently infected T-cells in vitro and the HDACi vorinostat significantly increase cell associated unspliced (CA-US) HIV RNA in HIV infected subjects on suppressive antiretroviral therapy (ART). However, it remains unclear if HDACi effectively induce viral protein expression or virion production, what proportion of viruses respond to HDACi activation and the effects of vorinostat on host gene regulation. These questions are important to address in order to determine the role of HDACi as a strategy to eliminate latently infected cells.

To understand the determinants of response of latent virus to an HDACi, we have recently developed a novel technique where integrated HIV LTRs from total memory CD4+ T-cells (CD45RO+) from patients on ART were amplified using triple-nested Alu-PCR. NL4-3 or patient-derived LTRs were then cloned into the plasmid pCEP4, which forms an episomal chromatin structure upon transfection. Quantification of luciferase expression was used to measure transcription under the control of the LTR. Constructs were transfected into Jurkat (T-cell), SVG (astrocyte) and Hela (epithelial) cell lines. We demonstrated equivalent response to activation of transcription in both T-cell and non T-cell lines and that several clones were non-responsive to HDACi stimulation. There were no signature sequences associated with nonresponse to an HDACi but many of these clones demonstrated a synergistic response to stimulation with an HDACi together with tat.

We also evaluated differential gene expression in blood from 20 HIV-infected patients on ART who received vorinostat (400mg/day) for 14 days. Gene expression was analyzed at baseline and two time points on vorinostat (d1 and d14) and post-cessation of vorinostat (d84). We found that the effect of

vorinostat on chromatin largely occurred within the 1st day after the first dose of drug and that after 14 days of continuous dosing, there were compensatory mechanisms associated with transcriptional repression and cell survival.

These results demonstrate significant effects of vorinostat on viral proteins and host genes that may have a significant impact on the potency of activation of latent virus.

ABSTRACT 53

Cyclic Dosing of Panobinostat to Reverse HIV-latency: Findings from a Clinical Trial

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BACKGROUND: Reactivating transcription of proviral HIV is currently investigated as a therapeutic strategy to eliminate latently infected cells and deplete the latent HIV-1 reservoir. We aimed to evaluate the ability of the highly potent histone deacetylase inhibitor (HDACi) panobinostat to reverse HIV latency.

METHODS: In a phase I/II clinical trial, HIV-infected adults on suppressive combination antiretroviral therapy (cART) received treatment with oral panobinostat 20 mg 3 times per week every other week over the course of 8 weeks while maintaining background cART. Patients were seen 13 times during the 8 weeks treatment period and at 4 and 24 weeks after completing panobinostat treatment. The primary endpoint was change from baseline in cell-associated unspliced HIV-RNA in CD4+ T cells. Secondary endpoints included safety, change in proviral total and integrated HIV-DNA, change in plasma HIV-RNA and change in the frequency of cells carrying replication competent virus. Histone H3 acetylation was measured on all study visits using flow cytometry.

RESULTS: Fifteen patients were included in the clinical trial. All patients completed full panobinostat dosing and follow up. Preliminary data in 13 of 15 patients revealed significant increases in HIV transcription as measured by levels of unspliced HIV-RNA in CD4+ T cells. Of 43 reported adverse events (AEs), 16 were presumed related to panobinostat; these occurred in 10 patients and were all grade 1 AEs. Fatigue was by far the most frequently reported AE presumed related to panobinostat treatment. Histone H3 acetylation levels increased significantly during periods of panobinostat administration, but returned to baseline levels at the post treatment follow up time points. Finally, detectable HIV-RNA in plasma was observed more frequently during panobinostat administration as compared to baseline.

CONCLUSION: Eight weeks of cyclic therapy with panobinostat was safe and well tolerated in HIV-infected adults on cART. Increases in cell-associated unspliced HIV RNA as well as increased detection of plasma HIV RNA were associated with panobinostat treatment indicating that this HDACi reverses HIV latency leading to increases in plasma HIV RNA.

ABSTRACT 54

Pharmacologically Induced Functional Cure-like Condition in Chronically SIVmac251 Infected Macaques is Associated with Immune Reconstitution and Broad Anti-Gag Immune Responses Increasing over Time

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BACKGROUND: We recently showed that a combination of highly suppressive antiretroviral therapy (H-iART), auranofin and buthionine sulfoximine (BSO) induced spontaneous post-therapy control of viral load in chronically SIVmac251-infected macaques [Shytaj et *al., Retrovirology.* 2013]. This control was associated with increases in anti-Gag immune responses and was dependent on the presence of CD8⁺ cells. We analyzed the long-term evolution of the viroimmunological parameters in these macaques.

METHODS: We analyzed samples from five chronically SIVmac251-infected macaques treated with H-iART (*i.e.* tenofovir, emtricitabine, raltegravir, darunavir/r and maraviroc), alone or in combination with auranofin and auranofin/BSO. Viral loads were quantified by real-time PCR and/or NASBA. Specific immune responses were detected by IFN- γ ELISPOT. CD4 counts were monitored by flow-cytometry following CBC.

RESULTS: All macaques displayed undetectable or low ($\approx 10^1$ SFC/10⁶ PBMCs) anti-Gag immune responses before therapy initiation. Long-term (up to 1 year) post-therapy follow-up of the macaques treated with a single cycle of H-iART/ auranofin/BSO showed increasing levels of anti-Gag specific immunity over time (up to >10³ SFC/10⁶ PBMCs; *P*<0.0001, *t*-test for slope). No significant increases were observed in the macaques that had not received BSO, or had received it

separately from the auranofin administration. The broadness of the immune response of the two macaques receiving a single cycle of H-iART/auranofin/BSO is shown by crossreactivity against SIVsm Gag (\approx 70% homology with SIVmac Gag). The macaque starting from the highest pre-therapy viral load set-point was subjected to an extended follow-up (1 year) and displayed a late viral set point (*i.e.* latest 5 months) of 1.7 Log_{10} vRNA copies/mL of plasma (*i.e.* an abatement of 4.7 Logs from pre-therapy levels). This was consistent with the continuing decrease of the viral set point and the continuing increase in CD4 counts during the follow-up (P=0.0007 and P=0.0003, respectively; *t*-test for slope) both paralleling the increasing anti-Gag responses.

CONCLUSIONS: The simultaneous administration of H-iART/auranofin/BSO results in the development and longterm maintenance of broad anti-Gag immune responses of increasing strength over time. This indicates that each of these interventions is fundamental for the induction of a functional cure-like condition with progressive improvement of viral load containment during the post-therapy follow-up.

ABSTRACT 55

Design and Delivery of Homing Endonucleases for Inactivation of HIV Provirus

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The development of highly active antiretroviral therapy (HAART) has led to significant increases in life expectancy and quality of life improvements for patients with HIV. Nevertheless, the long-term persistence of latently infected HIV reservoir memory T cells means HAART treatment must be lifelong, and the need for an HIV cure remains. As a novel approach towards an HIV cure we have targeted the HIV provirus for gene disruption in order to inactivate HIV and prevent reactivation from latently infected cells. Designer

homing endonucleases (HEs) that create double strand breaks (DSBs) at specific sequences within the HIV provirus can be used to introduce deletions and frame shift mutations within essential HIV genes upon imprecise non-homologous end joining repair of the induced DSB. We have used the HE I-OnuI as a scaffold to engineer HIV specific enzymes and have used a series of rationale and library-based approaches to enzyme engineering. Specific sequences within essential HIV genes that show similarity to wild type HE binding sites and with low levels of heterogeneity within HIV isolates were chosen as targets for enzyme selections. Using computational design, enzyme-DNA contact dependent amino acid randomization, and a yeast surface display selection assay we have developed variants of I-OnuI with specificity for sequences present in the HIV protease and integrase genes. Using a combination of designer HEs that target multiple essential HIV genes for disruption and subsequent inactivation we aim to inhibit the process of HIV inactivation from latently infected reservoir cells. We have tested this hypothesis in latently infected primary central memory T cells (Tcm), by delivery of designer HEs using scAAV vectors that can infect more than 90% of primary cultured Tcm.

ABSTRACT 56

Novel CD4-based Chimeric Antigen Receptors as Immunotherapy for an HIV Functional Cure

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BACKGROUND: Chimeric antigen receptors represent a promising strategy for functional cure of HIV. We engineered three CARs containing identical intracellular signaling domains (CD28 + CD3 ζ) linked to extracellular derivatives of CD4 (D1D2). These included CD4 alone (CD4 CAR) or attached to the 17b scFv targeting the highly conserved coreceptor binding domain, using either a long (35 aa; CD4-35-17b) or short (10 aa, CD4-10-17b) linker. Previous studies indicated that the corresponding soluble bifunctional proteins containing a long linker (sCD4-35-17b) neutralized HIV-1 strains with extremely high potency and breadth due to simultaneous binding of both sCD4 and 17b moieties to the same gp120 subunit; a protein with a linker too short for simultaneous binding (as in sCD4-10-17b) displayed only modest potency, comparable to sCD4 alone. Based on alternative concepts regarding the influence of CAR binding affinity, we sought to compare the relative functional activities of these 3 CARs.

METHODS: T cells were transduced with the CAR constructs and tested for anti-HIV activity using various *ex vivo* assays.

RESULTS: CD8 T-cells modified by each CAR were functionally activated upon Env engagement to secrete IFN- γ secretion, kill Env⁺ targets, and block HIV production by activated CD4 cells from infected subjects. All three CARs effectively suppressed HIV-1 infection of PBMC; the sCD4-10-17b CAR was significantly more potent than the CD4 CAR, and much more potent than the sCD4-35-17b CAR. These results support a model whereby T cell killing efficacy is compromised when the affinity between effector and target cell molecules is too high to permit "serial triggering". Strikingly, the CD4 CAR rendered CCR5⁺ cells highly susceptible to HIV-1 pseudovirus infection, an undesireable effect not observed with the bifunctional CARs due to both coreceptor binding site blockade and steric hindrance by the 17b scFV.

CONCLUSIONS: The novel CD4-10-17b CAR design offers both superior potency and safety.

ABSTRACT 57

Evaluating CTL-based "Flush and Kill" HIV Eradication Strategies Against Primary Cell Models of Latency and Natural HIV Reservoirs

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BACKGROUND: Purging of HIV-infected resting CD4⁺ T-cells is likely to require the co-ordination of latencyreversing agents (LRAs) with cytotoxic T-lymphocytes (CTL). We have developed an assay that incorporates HIVspecific CTL as sensors of latency reversal, and used this to assess LRAs in primary cell models. Based on our findings, we prioritized "flush and kill" strategies and tested these for eradication of HIV from natural reservoirs.

METHODS: CD4⁺ T-cells were depleted of activated cells and infected with HIV. Autologous HIV-specific CTL clones were co-cultured with these targets in the presence or absence of LRAs. IFN-gamma was quantified as a measure of CTL recognition. Optimal combinations of CTL and LRAs were co-cultured with autologous CD4 (no exogenous HIV) for 96-117 hours. CTL and dead cells were depleted and the residual reservoir was measured by qPCR and viral outgrowth assays.

RESULTS: We found that, while HDAC inhibitors (HDACi) and common gamma-chain cytokines both reversed HIV latency, HDACis suppressed CTL function and thus performed poorly in integrated "flush and kill" assays. In contrast, IL-15 superagonist (IL-15SA) both reversed HIV latency and enhanced CTL function. Treating patient CD4⁺ T-cells with a romidepsin pulse/wash followed by co-culture with HIVspecific CTL and IL-15SA resulted in a 5-fold reduction in levels of provirus and in the elimination of infectious virus as measured by viral outgrowth assays. CONCLUSIONS: There are advantages to incorporating CTL into early screening of LRAs for use in eradication strategies. Firstly, CTL can be used as sensors to detect latency reversal. This approach intrinsically incorporates a threshold of expression that is relevant to CTL clearance. Secondly, this allows consideration of the effects that LRAs may have on CTL function. Optimal LRA/CTL combination approaches can deplete the reservoir *in vitro*. Further study will determine the minimal elements required for this depletion, and the nature of residual HIV proviral DNA.

ABSTRACT 58

Ex-Vivo Expanded Cytotoxic T Cell Lymphocytes Enhance Clearance of Latent HIV Infection

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BACKGROUND: The immune system will play a key role in elimination of reactivated latent reservoirs; however, HIV infection renders HIV specific CD8+ T cells dysfunctional. Exvivo expansion of viral specific cytotoxic T cell lymphocytes (CTLs) derived from HIV infected patients may enhance the immune response to reactivated latent infection.

METHODS: T cells from HIV-infected patients on suppressive therapy were stimulated with autologous dendritic cells and PHA lymphoblasts loaded with overlapping peptides spanning consensus regions of HIV-1 gag p24, pol, and nef. To assess viral inhibition ability, autologous targets infected with JR-CSF virus or autologous reservoir virus (virus recovered from resting CD4+ cells in a viral outgrowth assay) were co-cultured with expanded CTLs or unexpanded CD8 cells. Supernatent p24 concentration was measured after 7 days and normalized to a control with no effectors added. Expanded CTLs ability to clear latent infection was evaluated using a novel latency clearance assay, in which resting CD4 cells were reactivated with maximal mitogen stimulation and co-cultured with expanded CTLs in replicate wells. Percent viral recovery was determined as number of p24 positive wells, normalized to the number of positive wells recovered with maximal mitogen activation in the absence of any effectors.

RESULTS: Following expansion, CTLs showed specific activity against HIV antigens in IFN γ ELISPOT assays, were polyclonal by flow-based Vbeta usage analysis, and cytotoxic in a chromium release assay against peptide pulsed targets. As compared to unexpanded CD8s, expanded CTLs reduced p24 production from autologous targets superinfected with JR-CSF (median %p24 produced with expanded CTLs=2.5%, vs 29.2% with unexpanded CD8s, p<.05) or autologous reservoir virus (median 8% vs. 20.6%, p<.05). Expanded CTLs showed a significant reduction in virus recovered from reactivated resting CD4 cells (median 50% recovered, p=.03) that was superior to the marginal, non-significant reduction seen with unexpanded autologous CD8 cells (median 90% recovered).

CONCLUSION: Ex-vivo expansion of CTLs derived from HIV infected individuals show superior in-vitro and exvivo anti-HIV activity as compared to unexpanded CD8+ T cells, including an enhanced ability to clear latent HIV infection. Ex-vivo expanded CTLs could thus prove useful in combination with latency reactivating agents in future studies.

ABSTRACT 59

Progress Towards the Clinical Validation of a Cytokine-enhanced pDNA Prime, rVSV Boost Therapeutic Vaccination Regimen Capable of Eliciting Robust, de novo, HIV-specific Immunity

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The advent of combination antiretroviral therapy (cART) has dramatically improved the clinical outcome in HIV-infected individuals through sustained reduction in viral replication. However, it is clear that cART alone cannot eradicate HIV in infected individuals due to the persistence of viral reservoirs in peripheral blood and tissue compartments. Consequently, a thrust of HIV research has been to develop therapeutic strategies that can boost host immunity to control viral replication upon discontinuation of cART (functional cure), or eliminate persistent viral reservoirs (cure). Therapeutic HIV vaccination is a key component in achieving these goals through vaccine-induced improvement in HIV-specific immune responses and increasing clearance of cells that harbor latent HIV.

Our approach to the development of an effective therapeutic HIV vaccine has been to clinically validate a plasmid IL-12 adjuvanted pDNA prime, recombinant vesicular stomatitis virus (rVSV)-vector boost vaccination strategy that has been shown to elicit robust, de novo, SIV-specific cellular immune responses in the SIV-infected, ART-treated rhesus macaque model. Using a step-wise clinical approach, the safety and immunogenicity of the plasmid IL-12 adjuvanted pDNA prime alone was first evaluated in normal healthy (IAVI B004) and HIV-infected (ACTG5281) volunteers, while the safety and immunogenicity of the rVSV vector alone was established in normal healthy adults (HVTN090). With the safety and immunogenicity of the individual prime and boost components established, clinical studies utilizing the full pDNA prime / rVSV boost vaccination regimen in normal healthy adults (HVTN087) and in HIV-infected volunteers treated during acute infection (Theravax 13-I-0141) are currently underway.

Preliminary blinded immunogenicity data from HVTN087 reveal that the prime/boost vaccination regimen elicited CD4+ and/or CD8+ HIV-specific cell-mediated immune responses in 5 of 6 (83%) vaccinated volunteers in the intermediate IL-12 dose group. In the Theravax study, the effect of vaccination on the rate of decay of the HIV-infected, CD4+ T-cell reservoir and the effect on rebound viremia following analytical treatment interruption in subjects who started therapy during acute or early HIV infection is also being directly addressed. An update on the clinical validation of the cytokineenhanced pDNA prime, rVSV boost therapeutic regimen and implications for the "cure agenda" will be presented.

ABSTRACT 60

Update in HIV Therapeutic Vaccines and Immunotherapy

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With the possible exception of "elite" controllers and the so called "Visconti patients" antiretroviral therapy (ART) need to be administered life-long to avoid an almost immediate (days or weeks) rebound of plasma viral load even after years of an apparently successful ART. The main reason is that the immune system is no longer able to contain a residual level of viral replication or the reactivation of latent infection. Conversely, the "recovered" immune is able to contain other infectious diseases and to respond to other recall antigens. The final scenario is that HIV is the only infectious disease needing life-long treatment. The cost and the burden for the health systems increase at least linearly since mortality is very low and the incidence of new infections still remain high.

The main objective of a therapeutic vaccine for HIV would be to avoid plasma viral load rebound after ART interruption, at least in a subgroup of patients and for a relatively long period of time if not forever. The most successful approach so far has been therapeutic vaccines based on monocyte derived autologous dendritic cells pulsed ex-vivo with whole inactivated (heat, chemicals) autologous virus or with electroporated mRNA of the autologous virus . Virological rebound is almost never prevented but the plasma viral setpoint achieved after ART interruption is ≥ 0.5 -1 log10 lower than expected in a proportion of patients. The magnitude of virological response correlates with HIV specific CD8 response. These therapeutic vaccine candidates may need to be improved or combined with adjuvants or with other candidates. Moreover, the HIV patients eligible for these therapeutic vaccine approaches may need to be better selected (eg. acute/early infection vs. chronic infection). Therapeutic vaccine candidates based on viral vectors (poxviruses, adenoviruses) have been less successful so far and in some cases the viral load rebound after ART interruption has even been higher than expected.

Finally, further studies are needed to assess if therapeutic vaccines may also be potentially useful to "overcome" the limitation of classical ART in terms of reducing residual viral replication, depleting the reservoirs or reducing the chronic inflammation or immune activation. Finally, if viral eradication would be an achievable goal we may need a combined approach including an intensified ART plus activating/mobilizing the latent viruses plus a therapeutic vaccine to kill the latently infected cells.

ABSTRACT 61

HIV Protected Zinc Finger Nucleases Mediated CCR5 Modified Autologous CD4 T-cells (SB-728-T) Reduce HIV Viral Load in CCR5 Δ32 Heterozygote Subjects During Treatment Interruption (TI): Correlates of Effect, and Effect of Cytoxan Pre-Conditioning Regimen

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BACKGROUND: SB-728-T treatment has been associated with prolonged increases in total CD4 counts. SB-728-T administration to a single CCR5 Δ 32 heterozygote HIV subject resulted in short-term control of HIV during a treatment interruption, possibly due to increase frequency of bi-allelically CCR5 modified CD4 T cells. We performed a prospective clinical study of studied HIV subjects who were CCR5 Δ 32 heterozygotes to confirm this finding. In order to determine if partial ablation improves engraftment, we performed a second study in which subjects were pre-treated with i.v. Cytoxan to enhance adoptive T cell engraftment.

METHODS: Two prospective studies were performed on HIV-infected adults: (1) CCR5 \triangle 32 heterozygotes (CD4 >500,

n=7), and (2) non-CCR5 Δ 32 heterozygotes pre-treated with Cytoxan pretreated (CD4 >500, 0.25-1 g/m², n=12). Standard clinical and laboratory safety was monitored. HIV viral load (VL), HIV-DNA level in PBMC by digital droplet qPCR, polyfunctional anti-GAG T-cell response by intracellular cellular cytokine staining and SB-728-T modified cell level by qPCR (pentamer duplication assay) were measured.

RESULTS: In seven SB-728-T treated CCR5 Δ 32 HIV subjects, VL decreased by >1 log from peak in three subjects during TI. Two subjects achieved unmeasurable VL, in one subject from 11 through 19 weeks of TI and ongoing. VL reduction from peak correlated with the level of circulating bi-allelically CCR5 modified cells during TI (r=-0.81, p=0.015). Subjects with reduced VL during TI had both a low HIV-DNA level at baseline (mean=63± 51 copies/ 10⁶ PBMC) and enhanced CD8 poly-functional anti-GAG responses after infusion with SB-728-T. Cytoxan pre-conditioning was well tolerated from 0.25-1 g/m² IV. Increases in circulating CCR5-modified CD4 T-cells, as well as total CD4 count post SB-728-T infusion appeared Cytoxan dose-related.

CONCLUSION: High levels of CCR5 modification, along with poly-functional CD8 anti-GAG responses, and low HIV-DNA levels in PBMCs appear to play an important role in functional control of HIV with SB-728-T treatment. Cytoxan pre-conditioning of HIV subjects appears safe and may enhance SB-728-T engraftment and total CD4 T cells. These results support further studies of immune effects of SB-728-T on suppression of acute VL.

ABSTRACT 62

Using an Autologous HIV Vaccine/ Activator (Based on the Full Length Virus Genome and the Intrapatient Virus Population) to Induce Latent HIV and Boost Immunity

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BACKGROUND: Complete eradication of HIV with antiretroviral drugs is almost insurmountable, as the virus persists in cellular reservoirs as latent proviral integrants. Most approaches to induce the latent HIV-1 pool involve some type of cell activation through mitogens, cytokines/chemokines, or HDAC inhibitors to up-regulate gene expression, which by default may also activate HIV-1 mRNA expression from latent proviruses. Prior to treatment, HIV-1 primarily infects HIV-specific CD4+ T cells that then transitions to the latently infected memory T cell population. Thus, we propose that the most effective and specific activator of latently infected T cells is the patient 's HIV-1 quasipecies prior to treatment.

METHODS: To test this hypothesis, we amplified the entire HIV-1 genome/population from plasma samples just prior to HAART then recombined the viral DNA population into a yeast-based vector. Defective, autologous virus vectors produced following transfections into 293T cells lacks the 5'LTR, the RNA packaging sites, the integrase active site, and the PPT. As an ex vivo test, this autologous HIV-1 vector (representing the patient's quasispecies prior to HAART), an NL4-3-based vector, or a cocktail of flu/tetanus/CMV antigens were loaded onto the DCs and then co-cultured with T cells (DC/T cells obtained from patients 3 years on stable HAART).

RESULTS: Based on 454 pyrosequencing, the autologous HIV-1 activator/vaccine had similar diverse viral population as that found in patients prior to treatment. In five different patients, the autologous HIV vector, presented by DCs, induced at least 30-fold higher HIV-1 production from the T cells than did the NL4-3 vector and 100-fold higher than the Flu/TT/CMV cocktail. In contrast, gamma interferon ELISPOTS on the DCantigen-T cell cocultivations revealed 10- to 100-times more spot forming units with the Flu/TT/CMV antigen cocktail than with the autologous HIV-1 vector.

CONCLUSION: These findings suggest that the entire intrapatient HIV-1 population in a safe, replication incompetent vector may be the best and most specific stimulus to drive HIV-1 out of the latent T cell pool. Finally, this autologous HIV-1 vector may also act as an immunogen to activate the HIV-specific memory T cells and to provide "new" help for both the humoral and CTL responses.

ABSTRACT 63

Dendritic Cell-based HIV Therapeutic Vaccination Increases Residual Viremia in Individuals On Antiretroviral Therapy

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BACKGROUND: We evaluated the impact of therapeutic vaccination with an autologous dendritic cell (DC) vaccine pulsed with autologous, inactivated HIV-1-infected apoptotic cells on the level of residual plasma viremia in individuals on suppressive antiretroviral therapy (ART) and following analytic treatment interruption (ATI).

METHODS: Autologous HIV-1 was obtained from 10 ART-naïve subjects prior to starting ART. The vaccine was composed of monocyte-derived DCs (matured with TNF α , IL-1 β , IFN γ , Poly I:C) pulsed with apoptotic cells generated from autologous lymphocytes infected with autologous HIV-1. After at least 12 weeks of virologic suppression (<50 cps/ mL), subjects received 3 doses of vaccine (10⁷ cells/dose, 2 weeks apart). Six weeks after the third vaccination, subjects underwent ATI with a booster vaccine dose given 2 weeks after ATI. Plasma samples from 3 timepoints pre-ATI and at 2 and 4 weeks post ATI were assayed for residual viremia by single copy assay targeting HIV-1 integrase (iSCA).

RESULTS: After at least 8 weeks of virologic suppression using the Roche Amplicor Assay v1.5, 6/9 subjects had residual viremia detected by iSCA ranging from 2.0-49.5 cps/mL. In 4/10 subjects, increasing levels of residual viremia were observed after vaccination despite continuous ART. In these 4 subjects, fold increase in residual viremia from prevaccination to immediately before ATI ranged from 2.9 to 41.8 (mean=14-fold increase). There was no corresponding increase in either CD4+ or CD8+ T cell activation (HLA-DR+CD38+) or a correlation between residual plasma viremia and CD4+ (r=-0.44; p=0.23) or CD8+ (r=-0.51; p=0.16) T cell activation. Two weeks after ATI, 8 subjects were undetectable by the Roche assay, but 6 of 8 had viremia detectable viremia by iSCA (range=19.9 to 3322 cps/mL; median=145.9 cps/mL). Plasma viremia measured by both assays rebounded to pre-ART levels by week 6 of ATI in 8/10 subjects.

CONCLUSION: Vaccination with an autologous DC-HIV vaccine increased residual viremia measured by iSCA in 40% of subjects despite continuous ART. This increase was not associated with increased T cell activation. iSCA is a more sensitive tool for detecting rebound viremia than the FDAcleared Roche Amplicor assay v1.5. Therapeutic vaccination may increase HIV-1 replication or expression from latent reservoirs.

ABSTRACT 64

HIV-1 Infection Abrogated by Drug-induced Reactivation of Apoptosis

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OBJECTIVES: Currently available antiretroviral therapy has met limited success in clearing HIV-1 infection. To develop a new strategy, we explored the ability of drugs to induce ablation of HIV-infected cells by activating apoptosis (programmed cell death), using monotherapy with two globally prescribed medicines. A similar approach – druginduced apoptosis – is widely applied in cancer therapy to eliminate malignant cells.

RESULTS: Although initially blocked by HIV-1, apoptosis can be selectively reactivated in HIV-infected cells by chemical agents that interfere with viral gene expression. We show that ciclopirox (a topical antifungal) and deferiprone (an iron chelator) activate apoptosis preferentially in HIV-infected H9 cells and in peripheral blood mononuclear cells infected with clinical HIV-1 isolates. In infected H9 cells, ciclopirox and deferiprone enhance mitochondrial membrane depolarization and initiate the intrinsic pathway of apoptosis - evidenced by caspase-3 activation, poly(ADP-ribose) polymerase proteolysis, DNA degradation, and apoptotic cell morphology. In isolate-infected peripheral blood mononuclear cells, the drugs collapse HIV-1 production to the limit of viral protein and RNA detection. Breakthrough did not occur despite prolonged monotherapy. Moreover, no viral re-emergence was observed after drug cessation. These findings are consistent with apoptotic elimination of the proviral reservoir. As expected from their clinical profiles, tests in mice and in human epithelial cell cultures revealed neither cell/tissue damage nor activation of apoptosis by these drugs. The drugs inhibit initiation of HIV-1 transcription (Hoque et al., *Retrovirology* 2009) and perturb both viral and cellular gene expression. We suggest that the apoptosis-based antiretroviral activity of these drugs stems from their ability to inhibit the hydroxylation of cellular proteins essential for apoptosis and for viral infection, exemplified by eIF5A.

CONCLUSIONS: HIV-infected cells can be eliminated through the therapeutic reclamation of apoptotic proficiency (TRAP) by ciclopirox and deferiprone. The drugs affect expression of specific cellular and viral genes. In a pilot clinical trial, treatment with deferiprone led to a decline in viral load without rebound (Hanauske-Abel et al., this meeting). These drugs therefore qualify as prototypes of selectively cytocidal antiretrovirals. Whether the strategy of apoptotic ablation can be combined with HIV-1 reactivation to achieve a cure remains to be seen.

ABSTRACT 65

In vivo Administration of Lithium does not Induce HIV-1 Reactivation or Changes in the Viral Reservoir

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BACKGROUND: It has been suggested that lithium, an inhibitor of Wnt signaling pathway, might synergize with HDAC inhibitors in inducing the reactivation of the latent HIV-1 LTR in a cell model of HIV-1 latency. We took advantage of a clinical study designed to assess the effect of lithium on HIV-associated neurocognitive impairment to explore its potential effect on HIV-1 reactivation and viral reservoirs. METHODS: Nine HAART-suppressed subjects diagnosed with HIV-associated neurocognitive disorders received treatment with lithium carbonate in tablets, beginning a 2-daily 400 mg dose, and changing further adjusting the dose according to drug levels in serum. Changes in total cell-associated HIV-1 DNA and RNA in circulating primary CD4+ T cells were estimated by droplet digital PCR at weeks 0, 2, 4 and 12 during treatment with lithium. The frequency of latently infected cells was also quantified by the viral outgrowth assay (IUPM) at weeks 0 and 12.

RESULTS: At baseline, the median total cell-associated HIV-1 DNA was 1,173 copies per million CD4+ T cells (IQR: 388-2343), and the frequency of cells releasing replicationcompetent virus following cellular activation was 1.6 IUPM CD4+ T cells (IQR: 0.5-3.1). Neither total HIV-1 DNA nor IUPM showed any statistically significant change at week 12. Likewise, quantification of the expression of HIV-1 RNA in CD4+ T cells did not increase after 12 weeks of treatment with lithium with a total median variation below 1% respect to the baseline. Of note, a transient decrease in the HIV-1 gene expression was observed at weeks 2 and 4, but normalized afterwards.

CONCLUSIONS: The therapeutic administration of lithium in HAART-suppressed subjects did not show any sign of viral reactivation or had a significant effect on the size of the HIV-1 reservoir.

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Ing-B (ingenol-3-hexanoate) is a Potential PKC Activator for the *Shock and Kill* Strategy in HIV Eradication

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BACKGROUND: Several potential HIV activator compounds have been suggested as coadjuvant therapy with cART for the eradication of latent reservoirs in HIV-infected patients. Here we present the preliminary results on a hexanoate derivative of Ingenol (Ing-B), a phorbol ester isolated from the Brazilian shrub *Euphorbia tirucalli*.

METHODS: In a pilot trial in SIVmac251-infected macaques, Ing-B was administered in escalating doses (1, 2.5, 5 mg BID) for one week each, alternated with off-treatment weeks. Blood samples were collected at 0, 3, and 7 days posttreatment for each time set and analyzed for chemical and hematological measurements, frequency of activated blood leukocytes (CD69+), plasma viral load, and PBMC pro-viral load. In another experiment, pigtailed macaques were dualinoculated with SIVDeltaB670 and SIV/17E-Fr and treated with cART. Blood and spleen biopsies were collected after three consecutive undetectable viral loads. For quantitative viral outgrowth analysis, resting CD4+ T-cells were isolated from PBMCs and serially diluted in duplicates. One set was kept as control while the other was treated with Ing-B for 10 days. Resting CD4+ T-cells were also isolated from spleen biopsies and seeded in AZT-containing media. Cells were kept untreated, or treated with Ing-B or PMA/ionomycin for 18 hours. Cells were then collected and viral DNA and RNA were quantitated by qPCR.

RESULTS: Ing-B activated blood leukocytes and increased plasma viral load in SIV-infected macaques. Accordingly, plasma viral load and activation markers decreased during the washout periods. In contrast to other phorbol esters, this novel compound was less toxic and can be orally administered to animals with no apparent side effects. In accordance with our *in vivo* data, Ing-B treatment of resting CD4+ T cells isolated from virus suppressed, SIV-infected macaques led to a significant increase in reactivated latent cells and viral RNA transcription.

CONCLUSION: We plan to treat virus suppressed, SIVinfected macaques with Ing-B to determine the ability of the compound to reactivate SIV *in vivo* and reduce the number of SIV latently infected resting CD4+ T cells, monocytes, and macrophages. Our data suggests that Ing-B is a viable candidate to be used concomitantly with cART for HIV eradication.

POSTER ABSTRACTS

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Platelets and Erythrocyte-Bound Platelets Bind Infectious HIV-1

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BACKGROUND: Despite effective antiretroviral therapy that suppresses HIV-1 RNA in peripheral blood, nonlatent reservoirs for HIV-1 still exist that cause low-levels of persistent viremia. Red blood cells (RBC) derived from infected patients have been reported to contain both cell surface-bound HIV-1 RNA and p24, and RBC have been hypothesized to represent an immunologically protected binding site for infectious HIV-1. However, HIV-1 has also been reported to be associated with platelets. Here we investigated whether infectious HIV-1 particles might be associated with platelets, or with RBC-bound platelets.

METHODS: RBC from citrated blood were collected from uninfected individuals, or blood with or without EDTA was collected from chronically infected HIV-1 patients. A small number of RBC having attached platelets was always observed by flow cytometry, light microscopy, and immunofluorescence microscopy. RBC, platelets, and RBC-bound platelets purified by FACS were incubated with HIV-1, washed, and coincubated with CD4-positive peripheral blood mononuclear cells (PBMC) to determine the presence of surface-associated infectious HIV-1 causing cell-cell infection. Plasma and RBC samples from patients were also co-incubated *ex vivo* with PBMC.

RESULTS: RBC either from uninfected or HIV-infected individuals invariably contained a small number of RBC having one or more attached platelets. After incubation with HIV-1, platelets, and platelet-RBC complexes, but not plateletfree RBC caused infection of PBMC. Infection was prevented by pre-treating the platelet-RBC complexes with EDTA. After incubation of cells or platelets with a monoclonal antibody to DC-SIGN, partial inhibition of cell-cell HIV-1 infection of PBMC was observed. All plasma samples obtained in the absence of anticoagulant from HIV-1-infected donors lacked infectious HIV, but platelet-RBC complexes from most of the patients caused cell-cell infection of PBMC. Infectious HIV-1 associated with the surface of platelets or platelet-RBC complexes was stripped from the cells by incubation with EDTA.

CONCLUSIONS: HIV-1 in blood is compartmentalized in that EDTA-free plasma from chronically-infected patients lacks infectious HIV-1, and platelets and platelet-RBC complexes, but not purified RBC, bind infectious HIV-1. Binding of HIV-1 to platelets and platelet-RBC is inhibited by EDTA; and DC-SIGN, and possibly other C-type lectins, might represent binding sites for infectious HIV-1 on the platelets and platelet-RBC complexes.

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ABSTRACT 68

A Previously Unidentified HIV-1 Reservoir, CD4⁺ Satellite, Plateletsized, T Cell-derived Bodies, Bind and Transmit HIV-1 Without Direct Virion Fusion

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BACKGROUND: We previously showed that CD4 counts are regulated by active α_1 proteinase inhibitor (α_1 PI, α_1 antitrypsin, Serpin A1) due to its motogenic properties during hematopoiesis. Under physiologic conditions, α_1 PI induces polarization of CD4, chemokine receptors, and functionallyrelated receptors on the plasma membrane, a condition that activates NF κ B and induces endocytosis of the receptor aggregate thereby promoting forward movement of the cell. We showed that this receptor aggregate is the preferred binding site for HIV-1 virions. In HIV-1 disease, a great proportion of α_1 PI becomes inactivated thereby limiting the ability of CD4⁺ T cells to migrate through hematopoietic tissue. To confirm these findings, we demonstrated that in HIV-1 individuals with >200 CD4 T cells/µl, α_1 PI augmentation therapy produced dramatically increased CD4 T cells in blood within 2 weeks of initiating therapy.

OBJECTIVE: In HIV-1⁺ individuals with <200 CD4 T cells/ µl, the relationship between α_1 PI and CD4 counts does not hold. We investigated the α_1 PI effect on CD4 T cells from this subset of individuals.

RESULTS: We show that in HIV-1⁺ individuals with <200 CD4 T cells/µl, as opposed to inducing endocytosis, a, PI induces the CD4 receptor aggregate to pinch off the plasma membrane forming satellite, platelet-sized, T cell-derived bodies (SPTBalls). Remarkably, the appearance of CD4+ SPTBalls in the blood of this subset of patients accounts in entirety for the depletion of detectable CD4⁺ T cells. To investigate SPTBall formation, we exposed whole blood, monocytederived dendritic cells, or U937 cells to α , PI for various times at 37°C and show using flow cytometry, confocal, and electron microscopy that that CD4 appears on SPTBalls after 60 min exposure of cells to a, PI. Because the HIV-1 preferred binding site is located on SPTBalls, we investigated whether SPTBalls are capable of harboring HIV-1. We show that SPTBalls are able to bind and transmit HIV-1 to activated, but not resting, primary T cells in vitro irrespective of the presence of the T-20 fusion inhibitor.

CONCLUSIONS: These results suggest that SPTBalls are capable of carrying out physiologic functions *in vivo* and may act as an HIV-1 infectious unit during sexual transmission. Importantly, SPTBalls represent a previously unidentified HIV-1 reservoir.

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Impact of an Intensified Highly Active Antiretroviral Therapy (HAART) Initiated during the Chronic Stage of SIV Infection on the Male Genital Tract

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BACKGROUND: Persistent shedding of HIV in semen has been described in a subset of men despite years of efficient antiretroviral treatment in blood, pointing at the male genital tract (MGT) as a reservoir for HIV/SIV. What remains unclear, however, is which organs/tissues of the MGT are responsible for this persistent shedding of virus? To this purpose, we studied the effect of a 4 month HAART on SIV infection and on the inflammatory response of the MGT.

METHODS: Male Cynomolgus macaques chronically infected with SIVmac251 were either left untreated (n=8) or given once a day for 4 months two RT inhibitors, tenofovir and emtricitabine, and the protease inhibitor lopinavir boosted with ritonavir (n=6). The treatment was intensified with an integrase inhibitor, raltegravir, over the last month. Blood and semen viral loads were regularly measured by RT-qPCR. SIV RNA and DNA were quantified in MGT organs and tissues using double amplification qPCR. The impact of HAART on MGT inflammatory response was investigated through quantification of a panel of 11 cytokines/chemokines mRNAs and of immune cell infiltrates.

RESULTS: The initiation of therapy rapidly decreased viremia in all animals. In semen, persistent SIV shedding was observed in 3/6 treated macaques. HAART had no major impact on viral RNA in urethra, nor on SIV DNA in any MGT tissues, whereas it significantly decreased viral RNA and DNA in the spleen. SIV RNA levels were significantly lower in the testis, epididymis, prostate and seminal vesicle of treated *versus* untreated animals. In fact, urethra and epididymis were the only MGT tissues displaying a median SIV RNA level above the detection threshold following HAART. Significant changes in various cytokines mRNAs levels were evidenced in all MGT organs tested. HLA-DR+ immune cell infiltrates were significantly decreased in the prostate and epididymis.

CONCLUSION: The treatment did not impact viral DNA levels in MGT tissues, or viral RNA in the urethra. These results, together with the phenotypic characterization of infected cells, the measurement of drug concentrations in MGT organs and the comparison of strain sequences in semen with that of MGT organs during HAART, currently under investigation, will be crucial to the further deciphering of the origin of HIV/SIV persistence in semen.

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Molecular Mechanism Governing Regulation of Resistance to Apoptosis to HIV Vpr Protein in Human Macrophages

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BACKGROUND: AIDS progression is characterized by rapid depletion of immune cells like CD4 positive T cells. Monocytic cells, however, survive HIV replication and consequent cytopathic effects because of their decreased sensitivity to HIV-induced apoptosis. HIV persists in these cells, shielded against various host-antiviral responses and anti-retroviral therapies. Monocytes and macrophages, therefore, are important HIV reservoirs and exhibit marked resistance to apoptosis upon infection, thereby hindering clearance of pathogens. However, the mechanism underlying the development of resistance to apoptosis in these cells is poorly uanderstood. METHODS: Primary human monocytes, monocyte-derived macrophages (MDMs) and THP-1 cells were stimulated with bacterial DNA or CpG. Unstimulated and stimulated cells were treated with Vpr-52-96 amino-acid peptide followed by the determination of mitochondrial depolarization and apoptosis by flow cytometry. The role of c-IAP-2, TRAF-1/2, Bax, Bid, caspase-8 and calmodulin-dependent protein kinase-II (CaMK-II) was determined by transfection of monocytic cells with their specific siRNA. The transfected unstimulated and bacterial DNA/CpG stimulated cells were treated with HIV-Vpr-52-96 followed by analysis of mitochondrial depolarization and apoptosis.

RESULTS: We have used primary human monocytes, MDMs and promonocytic THP-1 cells as a cell model to understand the resistance to apoptosis induced by HIV Vpr protein, a HIV accessory protein. We have demonstrated that primary monocytes and THP-1 cells when exposed to Vpr peptide are highly susceptible to mitochondrial depolarization and apoptosis. The mechanism involved in Vpr-induced apoptosis is not well understood. We have shown that Vpr-induced mitochondrial depolarization and apoptosis is mediated by tumor necrosis factor receptor-associated factor-2 (TRAF-2) degradation and subsequent activation of proapoptotic caspase-8, bid and bax. We also show that primary monocytes and THP-1 cells stimulated with bacterial DNA or CpG develop resistance to Vpr-induced mitochondrial depolarization and apoptosis. To understand the molecular mechanism governing such resistance to apoptosis, our results show that prior treatment with CpG or E.coli DNA prevented TRAF-2 down regulation, activation of caspase-8, bid and bax and subsequent mitochondrial depolarization and apoptosis by induction of antiapoptotic c-IAP-2 gene via CAMK-II activation in human monocytes and THP-1 cells. Furthermore, bacterial DNA-induced c-IAP-2 protects against Vpr-mediated mitochondrial depolarization/apoptosis by preventing the release of apoptosis-inducing factor and cytochrome-c through CAMK-II activation. In contrast, MDMs were resistant to such Vpr-induced effects that were attributed to cIAP expression as its inhibition by siRNA technology or Smac mimetics caused susceptibility to Vprinduced apoptosis in macrophages.

CONCLUSIONS: These results suggest a key role played by bacterial DNA-induced c-IAP-2 in preventing Vpr-mediated mitochondrial depolarization and apoptosis through prevention of TRAF 1/2 degradation and sequential inhibition of caspase 8, bid and bax. Furthermore, strategies based on suppressing c-IAP-2 by targeting CaMK-II may be helpful in selectively eliminating HIV persistence in macrophages and controlling reservoir formation.

ABSTRACT 71

Monotypic, Fully Functional HIV-1 Nef Persists in the Gut Mucosa of Aviremic Subjects on Therapy

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BACKGROUND: HIV RNA is detectable in the gut mucosa of 20-30% of individuals who are fully suppressed on therapy. Whether this is due to incomplete activity of antiretroviral(ARV) medications or due to a functionally distinct population of quasispecies with a survival advantage or some other mechanism is not known. The HIV-1 Nef accessory protein plays a key role in pathogenesis *in vivo* through numerous functions which may contribute to HIV persistence in the gut. We have previously demonstrated that HIV-1 Nef is compartmentalized in gut mucosa, both by sequence and function, and hypothesize that viral persistence in the gut despite suppressive therapy is in part due to the differential function of the compartmentalized *nef* alleles. Therefore we sought to compare Nef in gut mucosa of virermic and aviremic subjects on therapy.

METHODS: Total RNA was extracted from sigmoid colon biopsies of viremic and aviermic subjects then used as template for *nef* RT-PCR. Multiple clones were sequenced, then bulk PCR products were cloned into a modified whole genome proviral vector. Pseudotyped reporter viruses carrying *nef* alleles from these subjects were used to infect permissive CD4+ established cell lines and CD4 and MHC Class I downregulation was measured by flow cytometry. RESULTS: *Nef* was amplified from 30% of samples from aviremic subjects. Sequence analysis showed that clones from aviremic subjects had identical amino acid sequences and nearly identical nucleotide sequences, while those from viremic subjects were quite diverse. Nef from aviremic subjects maintained full function to downregulate CD4 and MHC Class I, while the majority of isolates from the gut mucosa of viremic subjects displayed significant impairment in CD4 downregulation.

CONCLUSIONS: Compared to viremic individuals, the *nef* alleles expressed in gut mucosa of aviremic individuals are essentially monotypic and remain fully competent to downregulate CD4 and MHC Class I. These results favor expression from reactivated latent reservoir cells rather than compartmentalized, low-level ongoing viral replication as the source of the persistent RNA expression while on therapy. However, further studies including analysis of other gene regions with a longitudinal design are required for a more conclusive result.

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Modeling Drug-privileged Lymphoid Sites: Necessary Conditions for Persistent HIV Replication

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BACKGROUND: HIV replication is suppressed below the standard limit of detection by Combination Antiretroviral Therapy (cART) in most patients. Recent results suggest that viral replication may continue in some patients, despite undetectable levels in the blood. Other work has suggested that lymphoid organs may be a site of reduced antiviral penetration and increased viral production. In this study, we create a mathematical model of reduced antiviral activity in lymphoid follicles, and explore the necessary conditions for persistent HIV replication, as well as the expected measured behavior of various markers in the blood.
METHODS: A simplified spatial model is presented modeling lymphoid follicles as disconnected diffusive regions joined through a large well-mixed compartment representing the blood and free-flowing lymph. Previously published experimental data is used to estimate the HIV reaction rates and the diffusion and transport rates within and between compartments. Monte-Carlo simulations are used to explore the dynamics of the system across the range of feasible values for the dynamic parameters.

RESULTS: Even in the complete absence of antiviral penetration into the lymphoid follicle site, only very limited viral replication can occur in lymphoid follicle sites with diameters of approximately 0.1 mm, consistent with un-inflamed lymphoid tissue. A bifurcation occurs at site diameters above 0.2 mm, allowing locally efficient, sustained HIV replication in the site that is not reflected measurably in the blood. When this locally efficient replication is perturbed by the addition of an integrase inhibitor, it produces transient, measurable peaks in 2-LTR artifacts as measured in the blood.

CONCLUSIONS: Reduced antiviral efficacy in lymphoid sites is not capable of producing significant levels of ongoing HIV replication with lymphoid site diameters consistent with un-inflamed lymphoid tissue. Site diameters above 0.2 mm, consistent with inflammation-induced hyperplasia, are capable of supporting locally efficient HIV replication if antiviral penetration is poor. Experimentally observed 2-LTR dynamics following raltegravir intensification can be generated by the disruption of this locally efficient replication.

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Next Generation Plasma HIV-1 RNA Single Copy Assay

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BACKGROUND: A qPCR assay with single copy sensitivity targeting HIV *gag* (gSCA) has been widely used to quantify persistence of low-level plasma viremia in the context of antiretroviral therapy (ART). However, mismatches between primers/probe and HIV *gag* sequences results in inefficient amplification in 15-30% of plasma samples with North American subtype B HIV-1. Additionally, recovery of the spiked internal standard (avian sarcoma virions) is often <50%, which is suboptimal. We therefore developed a next generation SCA by optimizing purification and targeting integrase (iSCA), which addresses these limitations.

METHODS: Primers/probe were designed to target the most highly conserved region of *pol* (3' region of integrase). Recovery of naked HIV RNA transcripts and spiked internal standard was assessed by qPCR to identify an optimal extraction method (sequential 3M GuHCl/Proteinase K, 6M GuSCN/glycogen, isopropanol precipitation). Paired plasma samples for comparison of gSCA and iSCA performance were collected from 30 consecutive subjects (25 with viremia suppressed on ART for >2 years; 5 with viremia >50 copies/ mL) through an IRB-approved protocol. The efficiency of gSCA and iSCA were compared to levels of viremia obtained with the Roche COBAS AmpliPrep/Taqman System (v2.0). HIV-1 RNA levels determined by gSCA or iSCA that were <10% of the Roche value were scored as inefficient amplification.

RESULTS: In the test panel of 30 paired plasma samples, median recovery of RCAS was 1.5 times greater with iSCA than gSCA (p<0.001, signed rank test). Of the 5 samples with viremia >50 copies/mL by Roche Taqman, gSCA efficiently amplified HIV-1 RNA from 2, whereas iSCA amplified HIV-1 RNA efficiently from all 5 patients (p=0.01, exact binomial test). For the 25 samples with HIV-1 <50 copies/mL,12 of 30

had greater than 3-fold higher recovery of HIV-1 RNA with iSCA versus gSCA (p<0.001, exact binomial test). With gSCA, 11 of 30 samples were below the limit of detection versus 7 of 30 with iSCA (p=0.088, exact binomial test).

CONCLUSIONS: This next generation plasma HIV-1 RNA single copy assay (iSCA) improves viral RNA recovery and reduces primer/probe mismatch such that inefficient amplification and false negative results are less frequent than with earlier assay versions.

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Quantification of Integrated HIV DNA by Repetitive Sampling Alu-HIV PCR and Poisson Statistics

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BACKGROUND: Proviral HIV is a candidate virological marker to monitor the HIV reservoir in patients. Recent data revealed that repetitive sampling Alu-HIV PCR on patient samples correlates with quantitative coculture assays, indicating that integrated HIV DNA correlates to the amount of replication competent HIV. Although promising, the workflow and data analysis of the assay is complex and hinders a wide use. Here, we provide an improved data analysis based on binomial and Poisson statistics analogous to digital PCR. This method enables absolute quantification without a standard dilution curve and includes confidence interval estimation.

METHODS: Poisson statistics were used to assess the binomial data of positive to negative reactions from the 42 replicate Alu-HIV PCR. Confidence intervals were calculated using a modified Poisson method, designed for data from \geq 40 replicates. The raw data of Alu-HIV PCRs on standard dilutions and on samples of HIV infected patients (N=47) were analyzed with the modified Poisson method and with the classical method of analysis (the % positive method) and these data were compared.

RESULTS: Both methods showed a linear correlation with the standard dilution series and a slope of about 0.1. This indicates that 10% of integrated viral DNA is actually amplified in the Alu-HIV PCR. An observation that is supported by previously published data. Hence, absolute quantification is possible with the Poisson method without a standard curve, but a standard sample as calibrator remains necessary for inter-assay and inter-laboratory comparison.

Assessment of the confidence intervals revealed that the new quantification method allows quantification at very low percent positive wells. In addition, the new method provides confidence interval assessment that define the minimal required number of technical replicates per sample.

CONCLUSIONS: The proposed methodology provides a more efficient workflow as preparation of the standard curve in the classical Alu-HIV is time consuming and requires frequent validation. In addition, the implementation of confidence intervals permits an improved qualitative analysis of the data and provides a statistical base for the required number of technical replicates.

ABSTRACT 75

Utilizing Antibodies as Indirect Markers for HIV Persistence in HIV Treatment Intensification and Eradication Studies.

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BACKGROUND: In HIV treatment and eradication protocols, measurement of success is difficult since viral load is very low and virus may remain sequestered in cells. Current methods for detection of persistent virus use large volumes of plasma or difficult to acquire patient specimens and are expensive. HIV "incidence assays" have been developed to measure antibody kinetics (titer, avidity, Ag-specificity) to identify individuals whose anti-HIV response is still evolving after seroconversion. These assays demonstrate antibody seroreversion during treatment as a consequence of reduction of antigenic stimulation from circulating virus and viral reservoirs. We hypothesized that these assays could be used in eradication and treatment intensification protocols as indirect markers of persistence of low level replication and viral reservoirs.

METHODS: We used HIV antibody diagnostics with or without modification to measure avidity, quantity and diversity of HIV antibodies. HIV incidence assays included: dilution (1:400 dilution in buffer; increased cutoff) and avidity modifications for the VITROS[®] Anti-HIV1+2 asay; an avidity (DEA-incubation/PBS-incubation; avidity index) modification for the BIO-RAD GS HIV-1/2 assay; and a calculated index summing HIV-specific band intensities p31, gp160, gp41 for the BIO-RAD Geenius HIV1/2 Confirmatory System. Using these methods we characterized anti-HIV responses from 280 long-term infected untreated individuals, 280 treated and virally suppressed individuals, 100 virally suppressed elite controllers and one potentially eradicated individual (the Berlin patient). We measured the decline of antibodies over time in long-term treated individuals and compared all groups.

RESULTS: In individuals who were treated after complete seroconversion, there were lower levels of anti-HIV reactivity when analyzed by time on treatment for the LS-VITROS (p=0.001) and Geenius (p=0.01). There were significantly lower levels of quantitative antibody parameters in HIVtreated compared to the untreated groups for all assays (p<0.001). There were lower levels of HIV antibodies among the elite controllers compared to the HIV-untreated group in the LS-VITROS, with less difference by the Geenius assay (p=0.01) and no difference using the Bio-Rad Avidity assay. However, elite controllers had higher antibody reactivity compared to ART-treated patients in all assays (p<0.001). The Berlin patient has low-level antibody reactivity that has progressively declined over time on these assays, consistent with lack of detection of virus.

CONCLUSIONS: There is a reduction in quantity, avidity and specificity of antibodies in treated compared to untreated individuals that correlate with time on treatment. Although elite controllers had undetectable or very low-level viremia, antibody levels remained elevated compared to treated individuals, demonstrating maintenance of antibody stimulation from persistent viral replication in reservoirs; however there was no change in the avidity of these antibodies. Measuring the quantity, quality and diversity of antibodies to HIV may be an ideal indirect marker for monitoring viral persistence in eradication and treatment intensification studies.

ABSTRACT 76

Quantitative Assessment of Persistent HIV Reservoir: An Alternative Approach to IUPM

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BACKGROUND: Strategies aimed at reactivation of latent HIV by manipulating epigenetic silencing mechanisms involved in the establishment and maintenance of HIV latency (such as Histone deacetylase inhibitors) are being tested for HIV eradication in clinical trials. The "gold standard" method to measure the frequency of latently infected cells is the quantification of infectious units per million (IUPM). This assay provides an accurate assessment of the latently infected cell pool ex vivo; however, it is time and cell consuming and lacks precision. We have developed a new assay to quantify the frequency of cells producing HIV multispliced (ms) RNA. While unspliced HIV RNAs are frequently detected in latently infected cells in the absence of viral production, HIV msRNA are correlated with active viral production.

METHODS: Purified memory CD4 T cells were stimulated for 12 hours with PMA/Ionomycin. Serial dilutions of stimulated cells were distributed in a 96 well plate (4 dilutions, 24 replicates) directly in RT-PCR buffer. msRNA was quantified by real time PCR, after semi-nested pre amplification, using primers that amplify specifically the spliced mRNA for tat/rev. The frequency of cells harboring inducible HIV msRNA was calculated using the maximum likelihood method.

RESULTS: We determined the size of the inducible HIV reservoir in aviremic treated and viremic subjects with a range of HIV integrated virus varying from 10 to 1000 HIV DNA

copy/million of CD4 T cells. We show a significant correlation between frequencies of cells with integrated HIV DNA and cells with reactivated HIV with 4 times more cells with integrated DNA in both viremic and aviremic treated subjects. Results from this assay also showed a positive correlation with the IUPM assay. This method is highly reproducible (less than 3% intra-operator variability) and has a sensitivity limit of 1.3 cells per million. It can be completed in two days and more importantly requires less then 1x10^6 target cells.

CONCLUSION: Our results show that this assay can be useful in providing an accurate quantitative assessment of the size of the functional HIV reservoir during ART; this will prove an important tool in evaluating the efficacy of reactivation strategies.

ABSTRACT 77

The Inducible Reservoir of HIV-1 in Resting CD4+T-cells Has Been Underestimated

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BACKGROUND: A major obstacle to curing HIV-1 infection is resting CD4+ T-cells (rCD4) harboring latent, replicationcompetent proviruses. Traditional assays of infectious virus recovery (IVR) from this latent reservoir involve a co-culture assay that relies on detection of p24 antigen production by robustly replicating viruses. Recent evidence suggests that the IVR assay detects only a small fraction of intact proviruses. Simpler and more sensitive measures of inducible HIV-1 reservoirs are needed.

METHODS: rCD4 were purified from blood of HIV-1infected donors on suppressive ART with plasma HIV-1 RNA <50 copies/mL for \geq 2 years. rCD4 were assayed in parallel for IVR and total virus recovery (TVR). TVR was measured by activating serial 3-fold dilutions of rCD4 in 10 replicates with anti-CD3/CD28 beads for 7 days. Pelletable virion-associated HIV-1 RNA in day 7 (D7) culture supernatants was detected using the Roche Taqman 2.0 and HIV-1 RNA units per million rCD4 (RUPM) were calculated by maximum likelihood estimate (MLE). IVR was measured based on p24 antigen detection and MLE (IUPM_{p24}). IVR was also calculated based on 10-fold increases in supernatant HIV-1 RNA between D7 and D21 (IUPM_{RNA}). Total HIV-1 DNA in rCD4 was assayed by qPCR.

RESULTS: All 10 donors had plasma HIV-1 RNA <20 cps/mL by Roche TM after a median of 9 years of suppressive ART. Median IVR at D14 was 0.5 IUPM_{P24} (range: <0.1-1.9) with 2 of 10 samples having <0.1 IUPM_{P24}. Median IVR using 10-fold HIV-1 RNA increases by D14 was 1.5 IUPM_{RNA} (range: 0.82-2.96), averaging 5.3-fold higher (range: 1-28.9) than IUPM_{P24}. TVR at D7 was detectable in all 10 donors with a median value of 9.4 RUPM (range: 0.6-23.1), which was 19.5- and 8.6-fold higher than IUPM_{P24} and IUPM_{RNA} at D14, respectively.

CONCLUSIONS: Total inducible virion recovery following activation of rCD4+T-cells from donors on suppressive ART is ~20-fold higher than infectious virus recovery measured by traditional co-culture. Part of this difference is attributable to underestimation of infectious virus recovery by p24 antigen detection. Total inducible virus recovery is a more sensitive measure of latently-infected cells capable of virus production.

ABSTRACT 78

Distinct Patterns of TLR-mediated HIV Reactivation in Latentlyinfected Microglial Cells and Monocytes

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ABSTRACT: Toll-like receptors (TLRs) recognize molecules derived from microbes and play a key role in mediating innate immune responses. Multiple TLRs are typically expressed in cells of the monocytic lineage, including microglia. Microglial cells constitute the major reservoir for HIV infections in the brain, where inflammatory conditions in the central nervous system (CNS) are believed to induce HIV-associated neurocognitive disorders (HAND). Using HIV-latently infected microglial cell lines, we investigated whether TLR stimulation can induce HIV transcription. Treatment with a panel of TLR ligands, including Mycobacterium tuberculosis (*Mtb*)-derived molecules, we found that, unlike in monocytic cells, reactivation of HIV by TLR ligands was significantly restricted in microglia. Flagellin (TLR5 agonist) and, to a lesser extent, lipopolysaccharide (LPS; TLR4 agonist) were able to reactivate HIV in hTERT-immortalized glial (hT_Huglia/ HIV) and in SV40-immortalized (CHME-5/HIV) human fetal microglial cells. By contrast, agonists for TLR1, 2, 4, 5, 6, or 8 (but not for TLR3, 7, or 9), potently reactivated HIV in THP-1/HIV cells and, to a lesser extent, in U937/HIV and SC/ HIV monocytic cells in an NF-kB-dependent manner. Mtbderived molecules PIM₆ and LprG, which are potent TLR2 agonists, reactivated HIV in THP-1/HIV, but not in U937/ HIV or SC/HIV cells which, unlike THP-1/HIV, showed no significant expression of TLR2. We conclude that TLR signaling probably plays only a minor role in activating HIV replication in the CNS, but can potentially drive replication in peripheral monocytic cells.

ABSTRACT 79

Novel Macrophage Latency Assay to Quantitate Myeloid Viral Reservoirs

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BACKGROUND: Despite the success of combined antiretroviral therapy (cART) in reducing viral loads, HIV eradication remains unachievable due to the presence of viral reservoirs in infected patients. Increasing evidence shows that infected monocytes are found in HIV-infected patients untreated and treated with HAART. In addition, monocytes traffic to the CNS and other tissues, such as spleen, differentiating into macrophages that become productively infected and spread the virus. Therefore monocytes not only represent a susceptible population for virus infection and replication but can also lead to viral reservoirs in the tissue where they differentiate into macrophages. METHODS: Our laboratory has developed and characterized an accelerated, consistent SIV infected pigtailed macaque model for HIV AIDS and neurologic disease. Using SIV infected pigtailed macaques treated with cART, we have demonstrated the presence of latently infected, resting CD4+ T cells in the blood and lymphoid tissues, this model is being used to characterize other potential cell reservoirs of HIV latency. In order to assess latency in monocytes and tissue macrophages in our SIV infected macaques, we developed an assay similar to the quantitative viral outgrowth assay used to quantitate HIV and SIV latent CD4+ T cell reservoirs. In addition, to ensure that there are no contaminating CD4+ T cells in the assay a PCR test has been developed to calculate the number of T cell receptor (TCR) transcripts in the assay.

RESULTS: Using this assay, we have quantitated the number of SIV-infected myeloid cells in peripheral blood and tissues of SIV-infected macaques. The results correlate with the viral loads and the severity of the terminal state of the macaques. In addition, the PCR test was used to successfully determine the number of cells expressing TCR after the selection. Finally, the latency assay is being used to quantitate the number of latently infected cells in peripheral blood and spleen in the current cART suppressed animals.

CONCLUSIONS: A latency assay was developed to quantitate the number of SIV infected myeloid cells in SIV-infected macaques. This assay was first optimized in untreated macaques and is now being used to quantitate latently infected macrophages in our cART-suppressed animals.

Searching for Viruses that Could Create a Latent Myeloid Reservoir

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BACKGROUND: Evolution of the tropism phenotype of HIV-1 is paramount to understanding HIV-1 pathogenesis, latency, and disease progression. There is a great deal of confusion about the entry phenotype of typical HIV-1 isolates. Recently, a new assay has been developed to define entry phenotype by the ability of HIV-1 to use a low density of the primary surface receptor, CD4. Infection of macrophages is associated with evolution of the viral Env protein to be able to utilize low densities of CD4 as a necessary step to become macrophagetropic. We have used this assay to identify infrequent examples of macrophage-tropic virus in the cerebral spinal fluid and genital tract. However the extent of macrophage-tropic virus abundance in other compartments, such as the blood, remains unknown. We set out to determine if macrophage-tropic viruses ever reach a point of systemic infection.

METHODS: Viral RNA was isolated from blood plasma samples from viremic subjects infected with either subtype B or subtype C HIV-1, and who were late in disease progression with CD4+ T cell counts of <100 cell/mm³. We performed single genome amplification to isolate individual *env* gene amplicons, which were cloned and analyzed for CD4 usage using Affinofile cells, a CD4-inducable cell line, in order to determine the viral entry phenotype.

RESULTS: To date, no examples of macrophage-tropic virus have been found in the blood. We validated that we are examining late stage subjects with the identification X4 lineages in over 50% of the subjects. We are continuing to expand the sample size of this analysis.

CONCLUSIONS: Macrophage-tropic viruses were previously considered to be major contributors to infection and disease progression. Recent availability of a more robust assay allows identification of viruses that have undergone the evolutionary step to utilize low levels of CD4 and allowed us to identify macrophage-tropic viruses as being rare and potentially limited in their evolution to specific compartments of the body. These results argue that there is unlikely to be a significant myeloid component to the latent reservoir, at least comprised of viruses that have evolved to enter cells using low levels of CD4.

ABSTRACT 81

Differential Role of Bromodomain Proteins in HIV-1 Latency

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BACKGROUND: Small molecules targeting bromodomain proteins, a well-conserved class of transcriptional regulators, have recently emerged as novel epigenetic therapeutics in hematological and virological disease. Bromodomains are helical interaction modules that specifically bind acetylated lysines in histones. We tested the effect of bromodomain inhibitors JQ1, I-BET, I-BET151 and MS417 on HIV latency. We and others showed that these compounds effectively reactivate HIV from latency in cell culture cells and select primary T cell models of latency. Our data indicate that bromodomain inhibitors activate HIV latency by a Tat-independent mechanism and implicate a thus far unrecognized bromodomain family member BRD2 in the establishment and/or maintenance of HIV latency.

METHODS: We performed a comprehensive lentiviral shRNA screen of BRD proteins in J-Lat cells to identify which BRD protein is involved in the regulation of HIV latency. We also performed coimmunoprecipitation experiments to test for interaction of P-TEFb and BRD proteins. RESULTS: We tested shRNAs against BRD1-9 in A2 and A72 cells and tested reactivation from latency. Knockdown of BRD8 resulted in activation of the HIV LTR, similar to BRD2 knockdown, however, this reactivation was still responsive to JQ1. In contrast, knockdown of BRD3 resulted in a decrease of basal HIV transcription, and the activatory potential of JQ1 was unchanged. Co-treatment of cells with JQ1 and *DRB*, a CDK9 inhibitor, resulted in a decreased ability of the cells to respond to JQ1 treatment. Similar results were observed when Cyclin T1 was knocked down, indicating that the JQ1 effect in latent cells requires intact P-TEFb.

In coimmunoprecipitation experiments we found that BRD2, which lacks a bone-fide P-TEFb interacting domain and has therefore not been associated with P-TEFb-binding, efficiently coimmunoprecipitated with the P-TEFb component Cyclin T1, an interaction that was enhanced in the presence of JQ1. This interaction also depended on intact acetylation sites in Cyclin T1, indicating that BRD2 interacts with P-TEFb via acetylated Cyclin T1 and the BRD2 bromodomain.

CONCLUSION: We report data that identify BRD2 and BRD8 proteins as new mediators of HIV latency and confirm BRD2 as a candidate target of bromodomain inhibitors in the reversal of HIV latency.

ABSTRACT 82

HIV-1 LTRs Derived from the CNS have Lower Transcriptional Activity and Fail to Bind Sp1 Protein

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OBJECTIVES/AIMS: HIV-1 enters the central nervous system (CNS) early after infection. As a relatively immune privileged compartment with compromised penetration of many antiretroviral drugs, the brain represents an ideal site for the establishment of a viral reservoir. We have recently shown that CNS-derived LTRs have reduced basal transcriptional activity in astrocytes and T-cell lines. Here we sought to determine the mechanism of reduced transcriptional activity in astrocytes and other CNS cellular reservoirs, and also the responsiveness of CNS-derived LTRs to Tat and HDACi in primary brain cell types.

METHODS: HIV-1 LTRs were cloned from CNS and non-CNS tissues (PBMC, lymph node, spleen) of patients on suppressive (n=3) and non-suppressive antiretroviral therapy (n=5). LTR sequences were analysed for activity using a luciferase reporter vector and transfection into astrocytes and T-cells. Transcription factor motif polymorphisms were identified using the transcription factor search engine database, TFSearch. Electrophoretic mobility shift assays (EMSA's) were used to determine the effects of Sp1 binding motif polymorphisms on the ability of the LTR core promoter to bind Sp1 protein.

RESULTS: LTR sequences isolated from the CNS had a significantly lower basal transcriptional activity in astrocytes (p=.0327) and T-cells (p=.002) compared to those isolated from the non-CNS compartment of the same patients. Sequence analysis of CNS-derived LTRs showed that >90% contained polymorphisms in the Sp1 binding motif. The majority of these mapped to the distal Sp1 site (site III), with polymorphisms also observed in sites I and II, and in sequences adjacent to- and intervening the Sp1 motif. All CNS LTRs demonstrated reduced Sp1 binding, the majority (70%) binding with an affinity 0.7 or lower of WT, 50% of these with an affinity of 0.5 or less. In contrast, 100% of non-CNS LTRs isolated from the same patients bound Sp1 with a similar affinity similar to that observed for WT (between 0.9 and 1.0).

CONCLUSIONS/DISCUSSION: Sp1 plays a critical role in the establishment and activation from latency. Our data suggest that unique transcriptional regulatory mechanisms involving altered Sp1 activity may exist within the CNS which could alter the responsiveness of HIV-1 infected brain cells to activators such as HDACi.

ABSTRACT 83

RNAseq Transcriptome of PBMC Treated with Ingenol B, a Novel HIV-1 Latency Reactivation Drug

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BACKGROUND: Ingenol B (ingenol-3-hexanoate) activates protein kinase C (PKC) inducing NF- κ B translocation into the nucleus promoting reactivation of HIV-1 latency with no cytotoxic effects in PBMCs cells and Rhesus monkeys. However, ingenol B increases cellular activation markers such as CD69 and HLA-DR in PBMC treated cells. The major goal of this study was to quantify the level of global gene expression and cytokine production (IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF- α and IFN- γ) in human PBMC from healthy donors treated with ingenol-3-hexanoate.

METHODS: Gene expression levels were assessed by transcriptome (RNAseq) using 10⁷ PBMC from healthy donor exposed to 0.32 µM ingenol B for 24h. Cytokine production was evaluated by BioPlex Magpix platform (Bio-Rad) using Bio-Plex Pro[™] Human Cytokine 8-plex Assay from culture supernatant of PBMC treated or not with 1µM ingenol B for 24h.

RESULTS: Ingenol B upregulated several genes related to transcription activation such as cyclin T1, TNF and p50 subunit of NF-kB. Moreover, important proteins for HIV-1 adsorption and entrance were downregulated such as CD4, CXCR4 and CCR5. Activation markers have not a similar profile. While HLA-DR was downregulated, CD69 and CD38 were upregulated with statistical significance. Ingenol B increase the expression levels of cytokines IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF- α and IFN- γ , with the follow fold change levels 12.37, 14.91, 19.29, 87.61, 3.66, 8.94 and 722.17, respectively. The cytokine upregulation was confirmed by the proteins levels evaluated by BioPlex Magpix platform. The upregulation of CDK-9 and cyclin T1 levels (PTEF-b components) were confirmed by immunoblotting of lymphocytic T CD4+ cells treated with ingenol B. CONCLUSIONS: Our results suggested that ingenol B activate several genes related to HIV transcription and down-regulate cell receptors genes important for virus entry supporting our results that this compound can also inhibit HIV transmission. The balance between pro-inflammatory and anti-inflammatory cytokines induced by ingenol B can explain its low cytotoxicity in both, *in vitro* and *in vivo* models.

ABSTRACT 84

Repression of HIV Transcription in Memory CD4⁺ T Cells by Blimp-1

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BACKGROUND: A barrier to eradicating HIV infection is targeting and eliminating latently infected memory CD4⁺ T cells, which are the main reservoir of latent virus. Our goal is to determine if T cell specific transcription factors restrict HIV transcription in specific T cell subsets to establish and maintain HIV latency. The transcription factor B Lymphocyte-Induced Maturation Protein 1 (Blimp-1) is expressed in B and T cells and upregulated in chronically HIV-infected patients. Blimp-1 inhibits IL-2 production which is regulated by a transcriptional element that shares similar properties to the HIV long terminal repeat (LTR). We hypothesize that Blimp-1 binds to the HIV LTR, inhibits HIV transcription and contributes to HIV latency.

METHODS: We used T cell lines and primary human CD4⁺ T cells to study the role of Blimp-1 in HIV transcription. HIV replication was monitored using luciferase reporters, RT-PCR, and HIV p24 ELISA assays. Binding of Blimp-1 to the HIV LTR was determined using chromatin immunoprecipitation assay. Blimp-1 expression was diminished using shRNA. The levels of Blimp-1 in different T cell subsets were measured with flow cytometry and immunoblots.

RESULTS: We show that Blimp-1 is expressed in primary peripheral blood CD4⁺ T cells and is further induced by T cell activation. However, infecting CD4⁺ T cells with HIV inhibits the induction of Blimp-1. Importantly, Blimp-1 is differentially expressed in T cell populations and in particular is highly expressed in memory CD4⁺ T cells which do not support robust HIV replication. Blimp-1 binds downstream of the HIV 5'-LTR in resting primary CD4⁺ T cells and represses Tat-dependent HIV transcription. Upon T cell activation with anti-CD3 and anti-CD28 antibodies Blimp-1 is released from the HIV LTR and this correlates with significant increase in HIV transcription in cells expressing Blimp-1, but not in Blimp-1-depleted cells.

CONCLUSIONS: We propose a model in which Blimp-1 acts as an HIV restriction factor in memory CD4+ T cells through binding downstream of the HIV LTR and remodeling chromatin. Intrinsic inhibitors of HIV transcription, such as Blimp-1, may provide novel therapeutic targets for purging latent virus from specific cells.

ABSTRACT 85

Exosomes from HIV-1 Infected Cells Carry Distinct Protein and miRNA Components Which Control Cell Survival and Apoptosis in Recipient Cells

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BACKGROUND: Recently, much interest has developed regarding mechanisms of extracellular delivery of nucleic acids and proteins among virally infected and recipient cells. While the role of exosomes in viral pathogenesis and disease states remains largely unknown, it is .30now widely accepted that exosomes play important roles in intercellular communication, cellular inflammation, antigen presentation, programmed cell death, and pathogenesis. HIV-1 encodes its own miRNAs that regulate viral and host gene expression. The most abundant HIV-1-derived miRNA, first reported by us and later by others using deep sequencing, is the TAR (Trans-Activation Response element) miRNA.

METHODS: Exosomes were purified from culture supernatants using multiple high speed centrifugations followed by opti-prep gradient. Exosomes were incubated with cell lines or primary cell for subsequent super infection with dual tropic virus.

RESULTS: We have recently found the presence of TAR RNA in exosomes from cell culture supernatants of HIV-1 infected cells and patient sera. We report that transport of TAR RNA from the nucleus into exosomes is a CRM1-dependent active process. Prior exposure of naïve cells to exosomes from infected cells increased susceptibility of the recipient cells to HIV-1 infection. Exosomal TAR RNA down regulated apoptosis by lowering Bim and Cdk9 proteins in recipient cells. We found 104 -106 copies/ml of TAR RNA in exosomes derived from infected culture supernatants and 10³ copies/ ml of TAR RNA in the serum exosomes of Highly active antiretroviral therapy (HAART)-treated patients or Long term nonprogressors (LTNPs). Very recently, we have found that TAR is able to activate cytokines in the recipient cells by increasing the nuclear accumulation of both p65 and p50 (component of NFkB complex).

CONCLUSIONS: Effect of exosomes on recipient cells may be related to a newly formed IKKb in the TAR treated cells which may be the result of TLR activation by TAR. We will discuss the effect of these biochemical steps in the recipient macrophage cells that result in alteration of cytokines which in part may explain the neuroinflammation observed in AIDS patients who are under HAART treatment.

ABSTRACT 86

Regulation of P-TEFb Activation and Proviral Transcription by Inducible Serine and Tyrosine Phosphorylation of CDK9 and HEXIM1

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BACKGROUND: Defining the RNA/protein interactions that hold the Tat co-factor P-TEFb within repressive 7SK snRNP and the distinct mechanisms by which P-TEFb is released from 7SK snRNP by Tat or T-cell activation signals are essential steps for understanding how HIV latency is regulated in resting T-cells.

METHODS: P-TEFb complexes were purified from T-cell lines with integrated proviruses, followed by protein characterization by tandem mass spectrometry (MS/MS) to identify post-translational modifications that could regulate P-TEFb release from 7SK snRNP and its recruitment by Tat to the HIV provirus.

RESULTS: MS/MS analysis of affinity isolated P-TEFb subunits (CDK9, CycT1, CycT2) and HEXIM1, which in the context of the 7SK snRNA inhibits P-TEFb kinase activity, led to the identification of numerous novel phosphorylation, methylation, and acetylation sites. Phosphorylation of Ser175 on the activation loop residue on CDK9 is strictly dependent on inducible PKC activity and the modified CDK9 is absent from 7SK snRNP. Mutation of Ser175 (S175A) completely blocked P-TEFb interaction with the bromodomain protein BRD4 leading to a robust Tat-dependent reactivation of proviral gene expression. The phosphomimetic S175D mutation caused a two-fold reduction in P-TEFb interaction with BRD4 while modestly enhancing association with Tat. This mutation also led to an enhancement in both basal and TCR-induced proviral gene expression albeit to a lesser extent compared to the S175A phenotype. Two phospho-sites, Tyr271 and Tyr274, situated within the C-terminal P-TEFb binding region of HEXIM1 were also identified by MS/MS

to be modified following PMA stimulation. Mutagenesis experiments indicated that phosphorylation of these tyrosine residues could result in release of P-TEFb from 7SK snRNP via loss of its association with HEXIM1.

CONCLUSIONS: The activation of P-TEFb activity in response to T-cell activation stimuli is a critical step required for the reactivation of latent HIV proviruses that is regulated by a series of post-translational modifications. Phosphorylation of HEXIM1 at Tyr271 and Tyr274 contributes to release of P-TEFb from 7SK snRNP while Ser175 phosphorylation of CDK9 plays an important role in mediating the competitive binding of Tat and BRD4 to free P-TEFb and provides an informative molecular marker for the transcriptionally active form of P-TEFb.

ABSTRACT 87

Endothelial Cell Stimulation Overcomes Restriction and Promotes Productive and Latent HIV-1 Infection of Resting CD4+ T Cells

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BACKGROUND: Highly active antiretroviral therapy (HAART) is able to suppress human immunodeficiency virus type 1 (HIV-1) to undetectable levels in the majority of patients, but eradication has not been achieved because latent viral reservoirs persist, particularly in resting CD4+ T lymphocytes. It is generally understood that HIV-1 does not efficiently infect resting CD4+ T cells, and latent infection in those cells may arise when infected CD4+ T lymphoblasts return to resting state.

METHODS: In this study, we co-cultured resting CD4+ T cells with endothelial cells and infected the T cells with pseudotyped X4 virus. RESULTS: We found that stimulation by endothelial cells can render resting CD4+ T cells permissible for direct HIV infection, including both productive and latent infections. These stimulated T cells remain largely phenotypically un-activated and show a lower death rate than activated T cells, which promotes the survival of infected cells. Effector memory T cells are preferentially infected and the stimulation by endothelial cells does not involve IL-7, IL-15, CCL19 or CCL21.

CONCLUSIONS: Endothelial cells line the lymphatic vessels in the lymphoid tissues and have frequent interactions with T cells *in vivo*. Our study proposes a new mechanism for infection of resting CD4+ T cells *in vivo*, and a new mechanism for latent infection in resting CD4+ T cells.

ABSTRACT 88

Influence of T Cell Maturation State on the Establishment and Maintenance of HIV Latency

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BACKGROUND: The major reservoir of HIV latency *in vivo* is resting CD4 T cells, predominantly of central memory (TCM) maturation phenotype. Because most clinical infections are caused by CCR5-tropic virus, it has been assumed that preferential HIV infection of TCM cells is due to this subset's associated expression of CCR5. We have addressed this supposition by examining whether the state of CD4 cell maturation itself is a primary determinant in establishment and maintenance of HIV latency.

METHODS: A unique T cell model was used to generate latent HIV infection directly in resting non-cycling primary CD4 cells through co-culture exposure to autologous activated CD4 cells, productively infected with NL4-3 (CXCR4-tropic clone). Latently infected cell subsets, representing the major T cell maturation phenotypes of naïve (TN), central memory (TCM), and effector memory (TEM), were isolated by flow cytometry cell sorting. Levels of integrated provirus in extracted high molecular weight cellular DNA were determined by quantitative real-time PCR analysis of HIV *gag* copies. Total replication competent virus (infectious units, IU) was assessed by expression of intracellular Gag, following cell stimulation with anti-CD3/anti-CD28. HIV reactivation by inhibitors of histone deacetylase (HDACi) was evaluated by droplet digital (dd) PCR quantification of viral *tat* and *gag* RNA expression.

RESULTS: All three maturational cell subsets contained integrated HIV DNA; but, TEM and TCM subsets had significantly more provirus than the TN subset did (10-fold and 3-fold, respectively). Recovery of replication competent virus paralleled the pattern of integration, with TEM and TCM subsets demonstrating 18-fold and 5-fold more IU than the TN subset. However, the relationship between levels of HIV integration and induction of replication competent virus was not constant between the different subsets. The ratio of IU to integrants was highest in TEM cells and lowest in TN cells, indicating that latent HIV may be more resistant to reactivation in TN cells. Preliminary experiments, using HDACi induction, showed a similar response pattern hierarchy among these cell subsets.

CONCLUSIONS: The maturational state of CD4 T cells significantly influences the establishment of HIV latency and its responsiveness to external inducers of reactivation. These findings have direct implications for treatment strategies in HIV eradication.

ABSTRACT 89

A Wake Up Call for HIV - Role of SETD6 Methyltransferase in Modulating HIV Latency

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Highly Active Antiretroviral Therapy (HAART) has successfully limited the replication and spread of human immunodeficiency virus (HIV). However, despite treatment, infection persists, mainly in resting CD4+ cell reservoirs, and once therapy is interrupted viral replication quickly rebounds. Extensive efforts are at present being directed at eliminating these infected cell pools. One promising approach, known as "Shock and Kill" involves the reactivation of transcriptionally silent provirus by activator compunds, followed by eradication of replicating virus by drug therapy and immune clearance. However, the efficiency of such applications is limited, and the significance of each of the molecular mechanisms that promote HIV into latency is still subject to debate. Among the pathways that lead HIV into latency, transcriptional repression as a result of low levels of the viral trans-activator Tat, and of the nuclear factor (NF)- κ B plays a major role in establishment of viral latency. Transcription interference and epigenetic constraints on histones that surround the integrated HIV provirus also impose HIV transcriptional shutdown.

In an attempt to reveal novel mechanisms that regulate NF- κ B activity on gene transcription, it was recently demonstrated that RelA is mono-methylated on lysine 310 (RelAK310me1) by a protein lysine methyltransferase SETD6. Methylated RelA serves as a scaffold for subsequent recruitment of the repressive histone methyltransferase GLP, leading to de-novo histone methylation and repression of RelA-target genes.

In this study, the role of RelA methylation by SETD6 in regulating HIV latency was investigated in cell line models for HIV latency. Upon modulation of SETD6 expression, HIV replication and occupancy of RelAK310me1, GLP and the H3K9me2, repressive marker on NF-κB binding sites of the integrated HIV promoter were determined. These experiments were also performed in the presence or absence of TNFa stimulation. Current experiments are confirming the role of SETD6 in primary resting CD4+ cells line models and in cells isolated from HIV infected patients. Overall, our study validates SETD6 as a target for reactivating latent HIV. It also positions the cross talk between protein methylation of NF-KB and histone modifications as a driving force that controls HIV latency. In the long-term, novel strategies that will reactivate latent HIV and lead to its complete eradication will be developed.

ABSTRACT 90

TRIM22 Genetic Variants Associate with Loss of Inhibition of HIV-1 Transcription and Advanced HIV-1 Disease

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BACKGROUND: Several host intracellular factors can influence the HIV-1 life cycle either before its integration in the host cell DNA or regulate proviral transcription and protein expression. In this regard, the large family of intracellular Tripartite motif-containing (TRIM) proteins, encompasses different members capable of interfering with HIV-1. In particular, TRIM22 is an interferon-induced protein that inhibits HIV-1 transcription and replication in vitro. TRIM22 shows marked amino acid diversity in humans suggesting that its variants may differentially affect HIV-1 transcription and virus replication and, potentially, influence disease progression. Two missense single nucleotide polymorphisms (SNPs), rs7935564A/G [asparagine(N)155aspartic acid(D)] (SNP-1) and rs1063303C/G [threonine(T)242arginine(R)] (SNP-2) characterize the coding sequence of human TRIM22 gene. We therefore tested whether these allelic variants had a differential impact on TRIM22 inhibitory effect on HIV-1 transcription and replication in vitro and whether they could be associated with a differential profile of disease progression.

METHODS: Renilla luciferase (Luc) activity was measured after infection with a recombinant HIV-1 of activated peripheral blood mononuclear cells (PBMC) isolated from 61 healthy seronegative blood donors. HIV-LTR-driven Luc reporter activity was tested in the presence of plasmid expressing TRIM22 variants. The SNP-1 and SNP-2 allelic discrimination was determined by real-time PCR in a cohort of 57 HIV-1+ individuals with advanced disease progression (AP) vs. 76 normal progressors (NP) and 95 Long Term-Nonprogressors (LTNP).

RESULTS: HIV-1 replication was more efficient in PBMC from donors with SNP-1G and SNP-2G than from those with SNP-1A and SNP-2C alleles. TRIM22 (T22)-AC, T22-

AG and T22-GC inhibited, whereas T22-GG enhanced basal HIV-1 LTR-driven transcription. *In vivo*, SNP-1G homozygotes and SNP-1A/G heterozygotes were more frequent in APs vs. LTNPs (Odds Ratio=2.072, p=0.005) or vs. NPs (Odds Ratio=1.809, p=0.022); in contrast, SNP-2G/C did not significantly associate with any state of HIV-1 disease progression. Although the SNP-2C/G distribution was similar among the groups, a GG haplotype was found more frequently in AP vs. LTNP (p=0.02).

CONCLUSIONS: *TRIM22* genetic diversity affects basal HIV-1 transcription *in vitro* and it is a potentially novel determinant of HIV-1 disease severity.

ABSTRACT 91

Macrophage Populations in the Spleens of SIV-infected Pigtailed Macaques and their Contribution to Virus Infection and Reservoirs

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When considering the potential for a functional cure for HIV-infected individuals, it is important to consider HIV infection of cells of macrophage lineage, particularly in tissues such as the brain where these cells are the primary reservoir of virus. Recent evidence suggests that macrophages in tissues constitute a self-replicating, renewable population not necessarily derived from peripheral blood monocytes. In addition, unlike lymphocytes, in which activation eventually leads to apoptosis, macrophages can become activated, replicate virus, and then return to a resting state, suppressing virus replication yet retaining the potential for future activation.

Distinct macrophage populations exist in tissues such as spleen, identified by unique patterns of surface marker expression. Macrophage diversity and their susceptibility to viral infection have not been fully investigated in the primate spleen. Using our SIV pigtailed macaque model of HIV infection, we identified and quantitated macrophage phenotypes and viral replication in these cells in the spleen during SIV infection.

Expression of CD68 (scavenger receptor associated with macrophage activation and phagocytosis) and Mac387 (marker for myeloid-related protein 14, which is associated with inflammation) increased during SIV infection. In contrast, expression of CD163 (a scavenger receptor responsible for the uptake of haptoglobin-hemoglobin complexes) was significantly downregulated at day 10 pi, but upregulated during late stage infection, particularly in macaques with more severe CNS disease. All 3 classes of cells were capable of replicating SIV, as determined by double labeling with immunohistochemistry and in situ hybridization. CD163 expression was highly correlated with levels of viral RNA in spleen, plasma, brain and CSF. Virussuppressive antiretroviral therapy beginning at 12 days p.i. reduced CD163 expression to preinfection levels, but CD68 remained elevated, suggesting residual activation in splenic macrophages. These data demonstrate the existence of a dynamic macrophage population in the spleen that changes in response to infection and contributes to SIV infection in spleen and potentially to systemic viral load.

ABSTRACT 92

Cell-Cell Transmission may Allow HIV to Modulate the Probability of Latency

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BACKGROUND: Cellular transmission of HIV occurs by two distinct modes. Virus may bud off the infected cell membrane and fuse with the membrane of the target cell (cell-free transmission). Alternatively, viral capsid proteins on the surface of the infected cell may mediate the formation of tight junctions and synapses between infected cells and uninfected target cells, and virus particles can be passed through the synapse (cell-cell transmission). In order for latency to occur, HIV transcription and translation must be suppressed. The viral protein Tat is a potent promoter of viral transcription, and stochastic transcription of HIV at low Tat numbers can result in "switch-like" behavior. We hypothesize that the large number of Tat molecules transmitted during cell-cell transmission reduces the probability of latency due to their combined promotion of HIV transcription.

METHODS: We develop a differential equation model of HIV replication and latency formation, including both cell-free and cell-cell transmission modes. We introduce terms describing the dependence of probability of latency formation that depend of the level of Tat present. Using previously published estimates of the various HIV dynamic rates, we explore how the optimal size of a cell-cell transmission bolus changes with the relative fitness of the virus.

RESULTS: Modulation of latency by cell-cell transmission can increase the steady-state viral load relative to a virus lacking the cell-cell transmission model. The strength of this effect depends on the baseline probability of latency (p) and on the basic reproductive ratio of the virus (R_0). Decreasing R_0 increases the ratio of steady-state viral loads between viruses exhibiting cell-cell transmission and viruses lacking cell-cell transmission. For large values for R_0 (greater than 10) and intermediate values of p, the cell-cell transmission mode has no selective advantage. For small values of R_0 and intermediate values of p, the selective advantage of cell-cell transmission can be as much as 30%

CONCLUSIONS: Cell-cell transmission may provide a selective advantage through the modulation of latency. This advantage increases with decreasing viral fitness, which may explain an evolution toward more synapse-forming virus after host immune responses reduce the viral fitness.

ABSTRACT 93

HIV Variants that Persist in Plasma on Suppressive ART Can Lead to Rebound After Treatment Interruption

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BACKGROUND: Combination antiretroviral therapy (cART) suppresses but does not eradicate HIV infection. Analysis of HIV RNA and DNA during suppressive cART has revealed that HIV includes populations of identical sequences, suggestive of clonally expanded populations, which are found in plasma, in peripheral blood mononuclear cells (PBMC,) as well as in anatomic compartments. HIV emerges rapidly after treatment interruption; rebound viremia also includes clonally expanded populations is unknown. To investigate the source of early rebound virus, we compared HIV present in plasma and cells prior to and following a short structured treatment interruption (STI).

METHODS: Stored plasma and PBMCs were obtained from patients (N=14) with viremia <50 c/ml on cART for \geq 1 y who were enrolled in a STI study. PBMC and plasma samples were obtained before and 7-14 days after STI. DNA extracted from PBMC and RNA from plasma were subjected to single genome sequencing (SGS) to amplify HIV p6-protease-reverse transcriptase sequences. Sequences were aligned with Clustal W, and underwent phylogenetic analysis using MEGA 5.2.

RESULTS: A total of 14 patients was sampled (median age 38.4 y, CD4= $610 \text{ cells/}\mu 138\%$, duration of suppression 2 y); all patients rebounded within 7-14 days of treatment interruption; 4 patients have been analyzed and 226 SGS obtained thus far. Rebound viremia consisted of both individual variants and

groups of identical sequences. In 2 of the 4 patients, clonal sequences present during rebound were identical to an HIV variant present prior to STI. In contrast, sequences from rebound viremia didn't match PBMC-derived HIV DNA; only 2/92 PBMC-derived sequences had a corresponding sequence emerge in rebound viremia; none of the identical sequences present in cells were present in rebound viremia.

CONCLUSIONS: Early rebound HIV consisted of individual and groups of identical sequences. Some identical sequences present in rebound viremia were present as single variants prior to STI, suggesting that rebound viremia emerges at least in part from expansion of low-level persistent viremia. Identical sequences identified in PBMC-derived HIV DNA were not represented in rebound viremia. Proviruses in PBMC do not appear to be a frequent source of rebound viremia.

ABSTRACT 94

CCR5 ∆32 Heterozygosity and HIV-1 DNA Reservoir Size in Individuals on Suppressive Antiretroviral Therapy

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OBJECTIVES: *CCR5*^{Δ32/WT} has been associated with lower preantiretroviral treatment (ART) plasma HIV-1 RNA levels and slower HIV-1 disease progression. Viral DNA levels have also been observed to be lower in *ccr5*^{Δ32/WT} patients compared to wild-type individuals, but prior investigations incorporated relatively small sample sizes and/or did not rigorously control for HIV-1 disease and ART factors. We therefore performed a case-control study of HIV-1 DNA levels in 291 *ccr5*^{Δ32/WT} and wild-type individuals on suppressive ART from the ACTG ALLRT cohort.

METHODS: Total HIV-1 DNA was isolated from PBMCs from participants in 4 ACTG trials of ART naïve individuals and quantified HIV-1 DNA normalized for a host gene using a sensitive real-time PCR assay. 291 samples were tested from 114 *ccr5*^{Δ32/WT} cases and 177 wild-type controls frequency matched in a 1:1.5 ratio by sex, initial ART regimen and time of study sample after ART initiation (160, 144, 128 weeks). Only samples from subjects who achieved viral suppression by 24 weeks after ART initiation without subsequent persistent low-level viremia (\geq 3 consecutive \geq 50 copies/ml), confirmed viral load \geq 200 copies/ml or viral blips \geq 1000 copies/ml were included. Conditional logistic regression models compared DNA copies (/10⁶ PBMCs or /10⁶ CD4+ T cells) between cases and controls, and after adjusting for pre-ART HIV-1 RNA and CD4+ cell counts.

RESULTS: Median pre-ART HIV-1 RNA was 4.8 \log_{10} copies/ ml for $ccr5^{\Delta 32/WT}$ cases and 4.9 for wild-type controls. Median pre-ART CD4+ cell count was 281 cells/mm³ for cases and 244 for controls. HIV-1 DNA levels were similar between $ccr5^{\Delta 32/WT}$ and wild-type individuals after 128-160 weeks of suppressive ART (median copies/10⁶ PBMCs = 61 and 69, respectively). There was no association between DNA copies (/10⁶ PBMCs or CD4+ T cells) and $ccr5^{\Delta 32/WT}$ [Odds Ratio (OR) for $ccr5^{\Delta 32/WT}$ =0.89 (95%CI 0.61-1.30), p=0.55 per \log_{10} higher DNA copies/10⁶ CD4+ T cells]. Results were similar after adjustment for pre-ART HIV-1 RNA and CD4+ cell counts [OR 1.00 (0.65-1.53), p=0.99 for \log_{10} DNA copies/10⁶ CD4+ T cells].

CONCLUSIONS: HIV-1 DNA levels were similar between ccr5^{Δ32/WT} and wild-type individuals, suggesting that persistence of cellular DNA is not associated with viral coreceptor expression after long-term ART.

ABSTRACT 95

HIV-1 DNA Decay Dynamics in Blood during More Than a Decade of Suppressive ART

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BACKGROUND: HIV-1 DNA decay dynamics and parameters associated with HIV-1 DNA levels during long-term ART are not well defined.

METHODS: Blood mononuclear cells obtained from patients during 7-12 years of effective ART were assayed for total HIV-1 DNA and 2-LTR circles by qPCR. Slopes of total HIV-1 DNA (log₁₀ copies/10⁶ CD4+ cells/year) were estimated by subjectspecific linear regressions. Plasma samples were assayed for residual viremia by qPCR.

RESULTS: 30 subjects were studied. HIV-1 DNA decreased significantly from years 0-1 and 1-4 on ART with median decay slopes of -0.86 (IQR: -1.05, -0.59) and -0.11 (-0.17, -0.06) log₁₀(copies/10⁶CD4+ cells)/year, respectively (p<0.001, p<0.001). Median decay slope was not significant for years 4-7 of ART (-0.017 [-0.061, 0.020]; p=0.09) or after year 7 of ART (-0.006 [-0.030, 0.015]; p=0.17). All subjects had detectable HIV-1 DNA after 10 years of ART (median 439 copies/106 CD4+ cells; range: 7-2074). Pre-ART HIV-1 DNA levels were positively associated with pre-ART HIV-1 RNA levels (Spearman=0.71, p<0.001) and with HIV-1 DNA at years 4, 7 and 10 on ART (Spearman r=0.75, p<0.001). There was strong negative correlation between pre-ART CD4+ cells and HIV-1 DNA levels at all 3 time points post-ART [Spearman r= -0.52 (p=0.003), r=-0.58 (p<0.001) and r=-0.51 (p=0.004), respectively], but this correlation was lost (r=0.09, p=0.63) after adjustment for pre-ART HIV-1 DNA levels. No associations were found (p≥0.25) between total HIV-1 DNA slopes and % activated (CD38+/DR+) CD8+T-cells (average during year 1-4) or residual viremia (n=18). 2-LTR circles were detected pre-ART in 20/29 and in 8/30 subjects at last follow-up.

CONCLUSIONS: Decay of HIV-1 DNA in blood is rapid in the first year after ART-initiation (86% decline), slows during year 1-4 of ART (23% decline/year), and plateaus after 4 years of ART (no further significant decline). HIV-1 DNA levels at plateau were directly correlated with both pre-ART HIV-1 DNA and plasma HIV-1 RNA levels. HIV-1 DNA decay is not associated with the levels of CD8+ T-cell activation or persistent viremia. The determinants of stable HIV-1 DNA decay and persistence require further elucidation.

ABSTRACT 96

Ongoing Decay of HIV 2-LTR Circles After Treatment Initiation with Raltegravir in Treatment Naive Patients

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BACKGROUND: Measurements of HIV RNA and DNA species are used to estimate the size and decay of the HIV reservoir. We measured HIV RNA and DNA after treatment initiation including raltegravir over three years to establish the decay dynamics in a unique cohort. Here we report the results of the extended observation period between week 24 and week 156 of this study.

METHODS: The PINT study was an open label, nonrandomized study to assess the quantitative characteristics of HIV RNA and DNA in patients who initiated therapy (raltegravir/emtricitabine/tenofovir) during either primary (n=8) or chronic (n=8) HIV infection. Single copy HIV RNA in plasma, as well as total and 2-LTR circle HIV DNA in CD4+ T cells were measured by real time PCR. Nonlinear mixed effects modelling evaluated components of decay.

RESULTS: After week 24, pVL decayed with a mean half-life of 1,008 days (standard error SE 580 days), which was not significantly different from no decay. Total and 2-LTR HIV DNA exhibited monophasic decay from week 24 onwards to week 156. The mean half-lives for total and 2-LTR DNA were 1,381 (SE 281) and 848 (SE 251) days respectively, significantly different to no decay but not from each other. pVL levels were more variable around the decay trend than both DNA species (T-test, p<0.04), and 2-LTR was more variable than total HIV DNA (p=0.03). This may indicate either higher variability of these molecular species due to ongoing rounds of infection and/or cellular reactivation, or be a reflection of different quantitation limits of the PCR assays. One patient failed treatment at ~130 weeks developing INT resistance mutations. This was not reflected by a simultaneous rise in total or 2-LTR HIV DNA levels.

CONCLUSIONS: The very low levels of plasma HIV RNA beyond 24 weeks and virtually no further decay, together with measurable ongoing decay of total and 2-LTR HIV DNA suggest that the measured t1/2 for these species are likely to reflect the decay of cells harbouring these molecules. 2-LTR circles may not be ideal markers for the detection of ongoing replication, even in the context of integrase inhibitor containing therapy.

ABSTRACT 97

Incomplete Adherence to Antiretroviral Therapy is Associated with Higher Levels of Residual HIV-1 Viremia

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BACKGROUND: The persistence of HIV-1 in the latent reservoir is reflected in the frequent detection of residual HIV-1 viremia below 50 copies/m despite long-term antiretroviral therapy (ART). Incomplete ART adherence is common even in individuals who maintain virologic suppression <50 copies/mL, but its effect on residual viremia is unknown. We hypothesize that incomplete ART adherence may lead to active viral replication and higher levels of residual HIV- 1 viremia, even for individuals with apparently successful virologic suppression.

METHODS: Medication adherence and residual viremia <50 copies/mL were quantified in participants of the REACH cohort of homeless and marginally housed individuals with HIV/AIDS. Participants (N=64) had at least 6 months of virologic suppression <50 copies/mL and were in the adherence monitoring cohort with unannounced pill counts on a random day at the participants' usual place of residence every 3-6 weeks. Residual viremia was measured by the singlecopy assay.

RESULTS: The median time of virologic suppression was 10.5 months [IQR 7.5-18.4 months] and the median average ART adherence over the prior 1 and 2 months was 94% [IQR 79%-100%] and 93% [IQR 82%-98%], respectively. Average ART adherence over the past 2 months was significantly associated with levels of residual HIV viremia (Spearman r = -0.25, P=0.04). One-third of participants with 100% ART adherence over the past 2 months had detectable residual viremia. On multivariate regression analysis, ART adherence over the past 2 months, but not duration of virologic suppression, CD4+ T cell count, or ART regimen, was significantly associated with levels of residual HIV-1 viremia. Detectable residual viremia was associated with virologic failure (>50 copies/ mL) on univariate Cox proportional hazard analysis (HR 2.08, P=0.02). However, on multivariate analysis, only ART adherence was associated with risk of virologic failure.

CONCLUSIONS: Detectable residual viremia observed in the setting of 100% ART adherence is consistent with ongoing release of HIV-1 from the latent reservoir. In the setting of incomplete ART adherence, a component of the plasma viremia may represent new rounds of HIV replication even when an individual's viral load is <50 copies/mL.

ABSTRACT 98

Dual Role of ING in HIV Replication: Inhibition of *De Novo* Infection and Activation of Viral LTR in CD4+ T Cells and Macrophages

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BACKGROUND: Current antiretroviral therapies successfully control viral replication in HIV-infected patients but are not able to eradicate viral reservoirs. Several compounds have been suggested for the reactivation of latent virus, including PKC activators and HDAC inhibitors. Here we describe the effects of three semi-synthetic phorbol esters extracted from the Brazilian shrub *Euphorbia tirucalli* - ING derivatives A, B, and C - in cell lines, and also in PBMCs, CD4+ T cells, and monocyte-derived macrophages (MDM) isolated from humans and macaques.

METHODS: The efficacy of ING B derivatives in activating HIV-LTR was assessed in five J-Lat clones by GFP expression, and also in U1, ACH-2, and J1.1 cell lines by p24 ELISA. Lymphocytes and macrophage were treated with the three compounds and analyzed for cell viability, surface marker expression, and proliferation by FACS.

RESULTS: All three derivatives equally activate the HIV LTR in all reporter cell lines. In J-Lat clones 6.3, 8.4, and 9.2, ING II compounds upregulated GFP expression in 20% of the cells. In J-Lat 10.4, however, more than 80% were GFP positive after treatment. In addition, p24 levels in U1, ACH-2, and J1.1 cells treated with ING derivatives were similar to those from cells treated with phorbol 12-myristate 13-acetate (PMA). ING B was slightly more active than A and C, and also less cytotoxic. Furthermore, a significant downregulation of HIV receptor and coreceptors was observed in lymphocytes and MDM from both humans and macaques, even when cells were treated at low concentrations of the compounds. Activation markers were upregulated in CD4+ T cells but not in MDMs, and no proliferation was observed in PBMCs. CONCLUSION: We conclude that derivatives of ING are able to efficiently upregulate HIV transcription while downregulating the expression of surface proteins essential for viral entry. These findings suggest that these compounds, in combination with antiretroviral therapy, could be a potential candidate for the "shock-and-kill" strategy by activating latent virus while limiting viral spread.

ABSTRACT 99

A Comparative Analysis of Clinically Available HDAC Inhibitors

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BACKGROUND: Antiretroviral therapy (ART) must be maintained for decades, as integrated HIV genomes capable of re-igniting viremia persist within resting CD4+ T-lymphocytes. Histone deacetylases (HDACs) act at the nucleosome-bound provirus to maintain latency, and our recent pilot clinical trial demonstrated that the HDAC inhibitor vorinostat can disrupt HIV latency in HIV+ aviremic patients. Other HDAC inhibitors that are pharmacologically more potent have recently entered testing as anti-latency therapeutic candidates, but the relationship between potency and clinical efficacy is unknown. The relative efficiency of these compounds in reactivation of replicative competent HIV from the resting CD4+ T-cells of aviremic patients is not known. We compared the ability of selected HDAC inhibitors to induce LTR expression and virus production using cell line models of latency and the Quantitative Viral Outgrowth Assay (QVOA), currently the gold standard assay to measure replication-competent virus.

METHODS: The HDAC inhibitors panobinostat, entinostat, romidepsin and vorinostat were tested in Jurkat T-cell lines encoding the enhanced green fluorescence protein (EGFP) within the HIV genome. EGFP production as a marker for LTR activity was measured by flow cytometry and toxicity measured by Alamar Blue assays. Viral recovery from purified resting cells obtained from aviremic, ART-treated HIV+ patients was then compared in limiting-dilution outgrowth assays using standardized, published methods.

RESULTS: Panobinostat and romidepsin robustly reactivated virus at concentrations that were 25 fold lower than vorinostat in Jurkat cell models of latency. However, while both panobinostat and vorinostat induced virus outgrowth from resting CD4+ T-cells at similar efficiencies, romidepsin allowed only inefficient viral recovery. Entinostat was also a weak inducer of virus production in both cell lines and resting CD4+ T-cells.

CONCLUSIONS: As HDAC inhibitors advance into clinical testing, understanding the efficiency of these drugs in reactivating virus ex-vivo may help prioritize and refine study design.

ABSTRACT 100

Provirus Excision by Expression of a HIV-1 LTR-specific Trerecombinase with Broad Anti-viral Activity

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BACKGROUND: HIV-1 integrates into the host chromosome and persists as a provirus flanked by long terminal repeats (LTR) that are transcriptionally inactivated in latently infected cells.

To date, established treatment regimens primarily target the virus enzymes, virus attachment or virus-cell fusion, but not the integrated provirus. Therefore, current antiretroviral therapies require lifelong treatment which, unfortunately, is frequently accompanied by the occurrence of substantial toxicities and/or the development of drug-resistant viruses. In addition, these therapies do not address latently infected cells.

However, current approaches to activate latent reservoirs, e.g. by HDAC-inhibitors, also do not suffice to clear the pool of latently infected cells, suggesting that supportive antiviral strategies are needed.

Previously, we engineered an experimental LTR-specific recombinase (Tre-recombinase) that can effectively excise integrated HIV-1 proviral DNA from infected human cell cultures and, subsequently, demonstrated highly significant antiviral activity in humanized mice, suggesting that customized enzymes might someday help to eradicate HIV-1 from the body.

METHODS : Here we report the generation of a HIV-1 Trerecombinase with broad activity, recognizing >90% of clade B clinical HIV-1 isolates in a highly specific manner.

RESULTS : Preclinical toxicity and antiviral studies *in vitro* and *in vivo* revealed a good tolerance profile as well as efficacy. In order to reach latently infected cells and/or to combine Tre-activity with purging attempts, different inducible vector systems and latency models have been established. The advantages and disadvantages of these strategies in the context of future clinical applications will be discussed.

CONCLUSION: The present data suggest that the HIV-1 clade B-specific Tre-recombinase may be a valuable component of future antiretroviral therapies of the post ART era that aim at virus eradication.

ABSTRACT 101

Decrease of HIV-1 Proviral Integration in Human PBLs by Targeting PKC and Lck

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BACKGROUND: PKC theta () is highly expressed in T cells, where constitutes a central component in T-cell activation. During TCR signaling, PKC residues T538 and Y90 are phosphorylated by MAP4K3 and LCK, respectively, regulating PKC catalytic activation and plasma membrane translocation. This initiates a cascade signaling that ends in NF-B and NFAT and subsequent activation of effector T cells. We reported previously that blocking selectively PKC reduced viral replication in CD4+ T cells. Now we analyzed the effect of specific PKC or LCK inhibitors on HIV-1 retrotranscription and proviral integration.

METHODS: PBLs activated with PHA/IL2 for 48h were infected with NL4-3_renilla, NL4-3_wt, or VSV-Env-NL4-3_LUC for 18h or 6 days. Viral retrotranscription and proviral integration were analyzed by TaqMan qPCR. Cells were pretreated with the inhibitors 2h before and during the infection. NF-B and NFAT activities were analyzed with Luciferase expression vectors. Lck-defective JJCaM1.6 cells were used as control.

RESULTS: 1) IC₅₀ and CC₅₀ were determined for each inhibitor by classic antiviral assays: CGX0471 and CGX1079 (PKC inhibitors, CompleGen) inhibited HIV-1 replication more than 7-fold (IC₅₀=11.18M and 12.86M) (CC₅₀>50M); LCK inhibitors (Merck) reduced HIV-1 replication more than 16fold (IC₅₀=1.19M; CC₅₀>37M and IC₅₀=1.00M; CC₅₀>50M). 2) PKC inhibitors exerted the inhibitory effect mostly at proviral integration (>2.5-fold), whereas LCK inhibitors acted earlier in the viral cycle, before retrotranscription (>9.5-fold). 3) All PKC or LCK inhibitors blocked PBLs infection with VSV-Env-NL4-3_LUC, ruling out that the effect was at the viral entry. Moreover, JJCaM1.6 cells were refractory to the infection of both NL4-3_renilla and VSV-Env-NL4-3_LUC. 4) Analysis by flow cytometry after CFSE staining showed partial restriction of T-cell proliferation by PKC inhibitors in response to PHA or SEA, whereas LCK inhibitors completely blocked T-cell proliferation, maintaining viability. 5) NF-B and NFAT activities were reduced with both PKC and LCK inhibitors.

CONCLUSIONS: PKC and Lck inhibition blocks T-cell activation, reducing HIV-1 retrotranscription and proviral integration. In early HIV-infected patients, decreasing the pool of activated CD4⁺ T-cells by using these inhibitors as punctual adjuvants of cART could prevent the activation of infected CD4⁺ T-cells and the proviral integration in resting CD4⁺ T-cells, reducing the reservoir size.

ABSTRACT 102

Farnesyl Transferase: a New Target for Eradication of Latent HIV-1 Provirus in Jurkat T-cells

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BACKGROUND: In order to remove patients from antiretroviral therapy the reservoir of latently infected cells needs to eradicated. Factors that are responsible for maintenance of HIV-1 latency and compounds that can reactivate latent proviruses were identified by independent genome wide shRNA library screens and screens of the Merck compound library. We found that farnesyl transferase (FNT) was a novel target identified by both approaches.

METHODS: Jurkat T-cells (2D10) were super-infected with a synthetic shRNA library from Cellecta Inc. (Mountain View, CA) containing a total of 82,500 shRNAs targeting 15,439 mRNA sequences. Cells carrying reactivated proviruses were purified by sorting after sequential passages and the shRNA sequences identified by next-generation sequencing and classified by systems biology tools. Simultaneously, a high throughput small molecule screen of over 3 million compounds performed at Merck identified numerous FNT inhibitors including MRK-13 (MW: 463.971), which synergizes with the HDAC inhibitor Vorinostat (SAHA) in proviral reactivation assays.

RESULTS: The FNT α and β subunits and their interacting molecules were found within the top 1 - 20% of the hits. Individual shRNAs were used to knock-down (KD) the α and β sub-unit of FNT. Both shRNAs resulted in re-activation of latent HIV-1 provirus within 3 days, however, when FNT β was knocked down ~90% of the population became activated after 10 days. Both Image stream and immunoblot analysis revealed that knockdown of FNT β does not activate the NF- κ B-p65 signaling cascade. However, mutations in the NF- κ B binding element block efficient proviral re-activation in FNT β -KD cells suggesting that FNT β -dependent activator complexes are recruited through the enhancer element. Inhibition of c-Jun N-terminal kinases (JNKs) by SP600125 potently blocked HIV expression in the FNT β KD cells, suggesting that this pathway is constitutively activated in the absence of FNT β . However, HIV expression was unaffected by the ERK inhibitor U1026.

CONCLUSION: FNT β modulates the HIV-1 latency maintenance program through a JNK-dependent pathway. We conclude that FNT, particularly FNT β , is a pharmacologically attractive target that can be exploited in the design of therapeutic strategies aimed at inducing proviral clearance or long-term silencing.

ABSTRACT 103

Drug-based Lead Discovery: The Novel Antiretroviral Profile of Deferiprone in HIV-1-infected Cells And Patients

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BACKGROUND: We hypothesize that HIV-AIDS is a protein hydroxylation-dependent disease : Deoxyhypusyl hydroxylase is essential for HIV-1 gene expression and infected cell survival; prolyl 4-hydroxylase is essential for T cell reconstitution-disrupting lymphoid fibrosis. Dissecting their catalytic mechanism, we identified deferiprone (DEF, Ferriprox[™]) as antagonist of either hydroxylase, thus enabling dual target inhibition in vitro. In HIV-infected cells, we noted absent DEF breakthrough; and dose-dependent on-DEF decline of HIV-1 RNA without off-DEF rebound, resulting from HIV-conditional cell death (Mathews et al., this conference). From culture we proceeded directly to a proofof-concept short-term trial. As 'minimal antiretroviral activity of DEF monotherapy' in patients we defined the HIV RNA decline observed with zidovudine monotherapy, i.e. $-0.3 \log_{10}$ [Schooley et al. (1996) JID 173: 1354-1366], which reduces the annual risk of progression to AIDS-related death by 25% [Modjarrad et al. (2008) AIDS 22: 2179-2185].

Methods: Double-blinded, placebo-controlled, pharmacokinetic-related pilot trial in treatment-naïve consenting HIV-positive volunteers, using discontinuation trial design (DTD) for response-guided off-drug analysis [Kopec et al. (1993) JCE 46: 959-971].

RESULTS: Individuals who achieved 150 µM DEF in serum ({A}: N=7) on either 33 mg/kg or 50 mg/kg three times daily oral deferiprone demonstrated virological effect during the seven day [D] treatment [Dlog₁₀ D7 vs. D1: -0.40±0.1 (median±SEM)]; 6 of 7 experienced an acute viral load decline $[Dlog_{10} D7 vs. D1: -0.42\pm0.05]$. By contrast, the cohort that did not achieve 150 µM in serum ({B}: N=7), lacked an acute virological effect [Dlog₁₀ D7 vs. D1: +0.03±0.06; {A} vs. {B}: P=0.025]. Viral decline correlated with the DEF threshold [P=0.015 (two-sided Fisher's exact test)]. By DTD, responders {R} maintained their on-DEF nadir post-treatment through 46 days monitoring [on-DEF D7 log₁₀ -0.42±0.05 vs. off-DEF D53 \log_{10} -0.30±0.1; *P*=0.6], whereas non-responders {NR} increased HIV RNA [off-DEF D53 \log_{10} +0.25±0.1; {R} vs. {NR}: P=0.008). After complete withdrawal, DEF maintained antiretroviral effect for 552 times its half-life of about 2 hours.

CONCLUSION: As in culture, DEF suppresses virion production *and* rebound in persons. Approved for certain thalassemia patients with iron overload, DEF displays zidovudine-like activity and is the lead for cytocidal antiretrovirals, which selectively kill HIV-1 infected cells.

ABSTRACT 104

Ingenol-3-hexanoate is able to Reactivate HIV-1 Latency

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BACKGROUND: Different molecular mechanisms are responsible for HIV latency including the internalization of NF- κ B into the nucleus. There are several agents proposed to reactivate latent HIV; however, most of them are associated with high cytotoxicity and suboptimal reactivation levels. Here we evaluate the activity of a new compound extracted from *Euphorbia sp*, Ingenol B (ingenol-3-hexanoate) to reactivate HIV latency *in vitro* and *in vivo* models.

METHODS: Reactivation levels and cytotoxicity were evaluated in J-Lat. The ability of Ingenol B to activate PKC and promote NF-kB internalization was performed by PKC-GFP isoforms and immunofluorescence. The dependence of NF-kB binding site was evaluated using luciferase expression vectors controlled by HIV LTR region. Next, we examined HIV reactivation in the presence of Ingenol B in latently infected resting CD4+ T cells. To this, CD4+ T cells were isolated, activated, and infected with a nonreplicating HIV (pNL4–3Luc). After infection, cells were allowed to return to a resting state in limiting amounts of IL-2. Rhesus monkeys chronically infected with SIV251 and treated with three doses of 7 days of Ingenol B (1, 2.5, and 5 mg BID) with washout of 7 days.

RESULTS: Ingenol B was a better candidate to reactivate HIV latency if compared with others activators (SAHA, Briostatin and PMA) with no cytotoxicity effects (reactivation levels up to 20% with 0.32 μ M). The Ingenol B promotes phosphorylation of PKC isoforms (α , δ and γ) and translocation to the plasmatic membrane followed by NF-kB internalization into the nucleus. The Ingenol B activation is dependent on intact NF-kB binding sites in LTR region of HIV. The compound was able to reactivate virus transcription (4-6 fold up) in primary HIV-infected resting cells reaching levels of 10-15 fold induction when combined with SAHA or HMBA. The two Rhesus monkeys presented normal levels of CBC and toxicological tests during all 3 doses of Ingenol B tested, with a 1 log increase of RNA virus load during the treatment.

CONCLUSION: Our results suggested the potential of Ingenol B as a new compound able to reactivate HIV latency *in vitro* and *in vivo* making its very appropriate for HIV cure strategies.

ABSTRACT 105

Novel Neuroprotective GSK-3β Inhibitor Restricts Tat-mediated HIV-1 Replication

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BACKGROUND: The implementation of new antiretroviral therapies targeting transcription of early viral proteins in post-integrated HIV-1 can aid in overcoming current therapy limitations. To date there are no transcription inhibitors that can effectively inhibit HIV-1 in infected cells.

METHODS: Using high throughput screening assays, we have previously described a novel Tat-dependent HIV-1 transcriptional inhibitor named 6BIO. The screening of 6BIO derivatives yielded unique compounds that show potent inhibition of HIV-1 transcription.

RESULTS: We have identified a second generation derivative called 18BIOder as an inhibitor of HIV-1 Tat-dependent transcription in TZM-bl cells and a potent inhibitor of GSK-3β kinase in vitro. Structurally, 18BIOder is half the molecular weight and structure compared to its parental compound 6BIO. More importantly, we also have found differential GSK-3ß complex present only in HIV-1 infected cells. In uninfected cells GSK-3β was present in a complex with a molecular weight of ~300 kDa, which is likely to be composed of either homo- or hetero-dimers of GSK-3ß in conjunction with possible chaperone proteins or other bound proteins. However, HIV-1 infected cells displayed an extended set of smaller molecular weight complexes in addition to the dominant ~300 kDa complex. 18BIOder preferentially inhibits this novel kinase complex from infected cells at nanomolar concentrations. We observed efficacy of 18BIOder in HIV-

1 replication inhibition in humanized mouse model at both 1.0 and 10 mg/kg as measured through reverse transcriptase activity from treated animals. Longitudinal measurements over a span of 29 days showed effective viral suppression at 1.0 mg/kg 18BIOder shortly after treatment. When left untreated the virus would resume replication, a comparable response to cART which require daily dosage for effective plasma control of viral load. Finally, we observed that neuronal cultures treated with Tat protein are protected from Tat-mediated cytotoxicity when treated with 18BIOder.

CONCLUSIONS: Overall, our data suggest that HIV-1 Tat-dependent transcription is sensitive to small molecule inhibition of GSK-3 β . The sensitivity is validated *in vitro* and *in vivo* using biochemical methods (detection of novel GSK-3 β complex) and in humanized mouse model.

ABSTRACT 106

The Effect of Heme Arginate on Reactivation of the Latent HIV-1

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BACKGROUND: Infection with Human immunodeficiency virus-1 (HIV-1) and the Acquired immunodeficiency syndrome is an invariably deadly disease that can be slowed down and stabilized by current therapeutic approaches. However, the available antiretroviral compounds can control only the replicating virus while it is currently impossible to eliminate the integrated, latent HIV-1 from the reservoir cells. We have previously described that heme arginate, a drug approved for human use in treatment of hepatic porphyria, can reactivate the latent provirus in synergism with phorbol myristate acetate (PMA) or TNF-[], while inhibiting HIV-1 replication during the acute infection.

METHODS: ACH-2 cells harboring integrated HIV-1 provirus were left untreated, treated with heme arginate or other compounds alone or treated with heme arginate or other compounds together with PMA, prostratin or TNF-[]. Tin protoporphyrin IX, N-acetyl cysteine or vitamin E were also added to certain samples. Twenty four and 48 h after

stimulation, the cells and supernatants were collected and analyzed by western blotting (p24 Ag, []-actin), ELISA (p24 Ag) or qRT-PCR (Gag, GAPDH, []-actin).

RESULTS: We have compared the action of heme arginate with the action of other selected compounds approved for human use. None of these compounds alone induced reactivation of the latent HIV-1 in ACH-2 cells, but they all strongly potentiated the effects of PMA, prostratin or TNFalpha on reactivation of the latent HIV-1. The reactivation was inhibited by antioxidants N-acetyl cysteine or vitamin E, and in some cases increased by inhibitor of heme oxygenase-1. A common mechanism underlying these effects thus seems to include an increased redox stress. Redox stress was shown to affect epigenetic mechanisms regulating gene expression as well as to activate redox-sensitive transcription factors. Therefore, we propose a model in which heme arginate or other redox-modulating agents induce chromatin remodeling, affect binding of specific transcription factors to HIV-LTR and allow HIV-1 expression.

CONCLUSIONS: In summary, heme arginate and the other selected compounds seem to affect reactivation of the latent HIV-1 by inducing an increased redox stress. These results may point towards a new direction in the latent HIV-1 reactivation and thus help to eliminate the pool of reservoir cells.

ABSTRACT 107

Reactivation of Latent HIV-1 in Central Memory CD4⁺ T Cells through TLR-1/2 Stimulation

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BACKGROUND: The existence of latent reservoirs of HIV-infected cells constitutes a major impediment toward viral eradication. The current thinking in the field is that a

combination of hypothetical drugs that reactivate latent viruses, with anti-retroviral therapy (ART), will be an effective approach toward viral eradication.

Toll-like receptors (TLRs) are crucial for recognition of pathogen-associated molecular patterns by cells of the innate immune system. TLRs are present and functional in CD4⁺ T cells. It is established that memory CD4⁺ T cells, predominantly central memory cells (T_{CM}), constitute the main reservoir of latent HIV-1. However how TLR ligands affect the latent reservoir in central memory CD4⁺ T cells is poorly understood.

METHODS: Naïve T cells are isolated from peripheral blood of healthy donors followed by exposure with aCD3/aCD28 antibodies in the presence of selected cytokines to produce cultured T_{CM} cells. These cells are infected with replication deficient HIV-1, and allowed to achieve a resting state. Using these latently infected cells we have evaluated the activity of TLRs agonist to reactivate latent HIV-1.

RESULTS: We have found that the TLR-1/2 agonist Pam3CSK4 leads to viral reactivation from latency in a model of latency based on cultured T_{CM} and in cells isolated from aviremic patients. In addition, we investigated the signaling pathway associated with Pam3CSK4 involved in HIV-1 reactivation. We show that the transcription factors NF[]B, NFAT and AP-1 cooperate to induce viral reactivation downstream of TLR-1/2 stimulation. Furthermore, viral reactivation is independent of increasing levels of cyclin-T1 in resting cells but it is dependent on pTEFb. Finally, Pam3CSK4 reactivates latent HIV-1 in the absence of T cell activation or proliferation as compared with antigen stimulation.

CONCLUSION: Our findings suggest that Pam3CSK4 and/ or the TLR-1/2 signaling pathway can be targeted toward future development of anti-latency strategies, either alone or in combination with others anti-latency drugs.

ABSTRACT 108

Promising Role of Toll-like Receptor (TLR) Agonist in Concert with Prostratin for Eradication of Latently HIV-infected cells

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BACKGROUND: Persistence of cryptic, replication competent but silent HIV provirus under anti-retroviral treatment still remains the major hurdle for HIV eradication. Reactivation of HIV transcription alone might not guarantee an efficient elimination of the latently infected cells *in vivo*. Thus, we hypothesize that triggering the innate immune system in concert with transcriptional enhancers will result in a Th1 supportive micro-environment, crucial to clear the latent reservoir.

METHOD: Cooperative compounds were screened on a coculture of monocytes derived dendritic cells (MDDC) with J-lat cells clone 9.2, at a ratio 1:10. The model was subsequently characterized using flow-cytometry, neutralizing antibodies, transwell as well as a library of kinase inhibitors.

RESULTS: Triggering of the NF- κ B pathway by Prostratin, supported by TLR8agonist, resulted in an enhanced reactivation of HIV production (3 fold) compared to the compounds alone.

This combinatorial approach led to a drastic maturation of the MDDC, outlined by an increase in the activation markers, HLA-DR, CD80, CD83, CD86 as well as a secretion of TNF and MIP-1α. Moreover, Prostratin significantly down regulated Dc-Sign expression without inducing a proapoptotic phenotype.

The model consisted of two-step inducer, where Prostratin generate HIV reactivation through transcription factor availability, as well as a maturation of MDDC, which is fully achieved by addition of TLR8agonist. In the second step, TNF and co-stimulatory molecules accomplished a second signaling event, re-enforcing the effect of Prostratin in the latently infected cells. CONCLUSIONS: These findings provide evidence for an enhanced combinatorial reactivation approach, which might be able to target different mechanisms of latency through the involvement of concomitant signaling pathway events. Moreover, an HIV-specific CTL response could be subsequently mounted to constraint the residual infected cells.

Studies are on-going to assess the concept *in vivo* in HIVinfected humanized mice.

ABSTRACT 109

Jatropha Sp. Extract Induces Internalization of Cd4 Receptor and Inhibits HIV-1 Entry

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BACKGROUND: Highly active antiretroviral therapy (HAART) has been used as protocol treatment to HIV-1 infection; however, virus resistance contributes to therapeutic failure. Therefore, new compounds that target different steps of replicative cycle could potentially inhibit resistant viruses and improve AIDS prognostic rates. Here, we screened extracts from the Brazilian plant *Jatropha sp.* to evaluate potential compounds with antiviral activity against HIV-1.

METHODS: Several extracts were obtained from *Jatropha sp* preparations in different solvents. The cytoxicity and antiviral activity were initially screened in TCD4+ lymphocytic cells (MT-4). To elucidate the role of *Jatropha sp.* extract in the HIV entry, cells were pre-exposed to increasing concentrations of extracts and the HIV infectivity was measured by luciferase reporter gene cloned in the HIV genome (HIV-NL4.3 Luc) and the internalization of CD4 receptor was checked by confocal microscopy and flow cytometer using specific antibodies against CD4. To elucidate the molecular mechanisms of CD4 internalization several mutants of CD4 tail harboring mutations in the sites related

to phosphorylation, ubiquitination, and binding of AP2 were transfected in HeLa cells treated with extract and the CD4 staining. CD4 degradation was evaluated by the lysosome inhibitor bafilomycin and immunoblotting.

RESULTS: The THS fraction decreased HIV-1 infectivity up to 80% in a dose-dependent manner with no cytoxicity. The maximum inhibition was observed at 260µg/ml of THS. THS inhibition was dependent in envelope proteins with no inhibition in HIV VSVG pseudotyped virus. THS treatment induces internalization of CD4 receptor in MT-4 and PBMC cells, direction to early endosomes, followed by lysosomal degradation. The CD4 internalization promoted for THS treatment was mediated by PKC activation and consequent phosphorylation of CD4 tail serine residues. Our results showed that the CD4 endocytosis is dependent on dynamin activity and recruits AP-2 protein.

CONCLUSIONS: Our results showed that THS extract from *Jatropha sp.* induce CD4 internalization and inhibit HIV entry suggesting that compounds can be potentially used as microbicide to prevent HIV transmission.

ABSTRACT 110

Reactivation of HIV Latency by Ingenol Esters Isolated from Euphorbia Tirucalli and Other Semisynthetic Derivatives

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BACKGROUND: During the early stages of HIV infections, the virus establishes latency in a portion of the population of infected long-lived memory cells CD4+. Such latent viral reservoirs are inaccessible to standard antiretroviral drug therapies and result in an inability to eradicate the infection. A reactivation of the latent virus would offer an opportunity to expose it to the action of antiretroviral drugs (HAART) and consequently lead to full elimination of the virus. We have found that esters of ingenol, a PKC agonist, found in E. tirucalli act reactivating HIV from latency.

RESULTS: Ingenol esters were isolated by column packed with silica gel 60 and after automated procedures were used, as the Centrifugal Partition Chromatography (FCPC Kromaton, Rousselet Robatel; France) followed by preparative HPLC. Their structures were characterized by NMR as three E/Z isomers of ingenol-3-dodecatrienoate (1): 2E, 4E, 6E- (1a), 2E,4Z,6E- (1b), and 2E,4E,6Z- (1c). Antiviral MT-4 assay shows an important viral inhibition by compound 1, until reaching toxic concentration at 40 µM. The assay in TZM-bl cells shows that this compound acted as an activator of the HIV promoter, since there was a dose response of luciferase activity with increased concentration of 1. Experiments demonstrated that 1 is able to reactivate HIV latency at high levels in JLat6.3 cells. Additionally, experiments with different luciferase reporter plasmids demonstrated that 1 was able to increase transcriptional activity (figure 3). Interestingly, this compound was also capable of activation of human CD4+ cells, as shown by double marked CD4/CD38 and CD4/CD69 (figure 4a, b and c). Nevertheless, 1 was not able to induce cellular proliferation (data not shown). By acting through proviral activation and by blocking viral entrance through receptor down regulation, 1 can be used in a shock-andkill strategy to be placed together with HAART therapy to eliminate viral reservoir . It was noticed that Ingenol esters extracted from E. tirucalli were very unstable and their isomers different were difficult of to dose them. Because of this instability, it was decided to test various molecules with differents radical using different size of carbon chains. Although, resulted in three molecules which were used in this study: ingenol A, B and C. We tested them in the ability to reactivate latent HIV in JLat model and cytotoxic effect in human PBMC. We found that Ingenol B was the compound less toxic (IC50%>100 µM) and more potent in reactivating HIV showing maximal HIV reactivation in concentrations as low as $0.34 \,\mu M$.

CONCLUSION: Our results demonstrate that 1 acts by activation of HIV LTR promoter and by down regulating of the HIV receptor. By acting through proviral activation and by blocking viral entrance through receptor down regulation. We showed that synthetic compounds derived from natural Ingenol esters were very effective and Ingenol B showed the best profile to be a useful PKC inducer candidate to be used in a shock-and-kill strategy to eliminate viral reservoir.

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ABSTRACT 111

A Phase 1 Study of the Safety, Pharmacokinetics and Antiviral Activity of BIT225 in Patients with HIV-1 Infection

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

METHODS: BIT004 is a placebo-controlled, randomized study of the safety, pharmacokinetics and antiviral activity of BIT225 in 21 HIV-1⁺, antiretroviral therapy naïve subjects. Subjects received BIT225 (400 mg BID) or placebo treatment for 10 days (randomized 2:1). Twenty-one subjects were enrolled and completed treatment. To explore the potential of BIT225 to reduce the viral burden within the monocyte reservoir, CD14⁺ monocytes isolated from the peripheral blood on days 0, 5, 10 and 20, were cocultured *ex vivo* with MT4 T cells. *De novo* HIV-1 replication was measured by p24 activity of released virus into the culture supernatant to day 25 of coculture. In addition, monocyte samples were collected for RT-PCR HIV-1 single copy assay analysis. RESULTS: BIT225 was safe and well tolerated in HIV-1⁺ treated individuals. Preliminary analysis indicates that C_{max} and AUC pharmacokinetic parameters of BIT225 in plasma were comparable to previous trials with BIT225, with an apparent terminal half-life >16 hours on day 10, suggesting that BIT225 may be suitable for once daily dosing. As expected no changes in viral load or the CD4⁺ T cell count was observed in the treated individuals. However, by measuring HIV-1 directly within the patient's monocyte population, BIT225 treatment significantly reduced the viral burden in those cells that was more evident in those individuals with high viral loads.

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

ABSTRACT 112

Some Dysregulated Immune Responses in Lymph Nodes and Gut during RT-SHIV Infection are not Restored to Normal after Suppressive ART

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BACKGROUND: Our previous studies have shown little or no evolution of RT-SHIV in macaques during suppressive antiretroviral therapy (ART). However, viral RNA can be detected at low levels in the blood and tissues during ART. Chronic inflammation and immune dysregulation can persist during treatment. To understand how immunity is altered during viremia and how chronic inflammation contributes to viral RNA expression and persistence, we characterized the expression of >120 genes associated with inflammation in lymph nodes (LN) and small intestine of uninfected, viremic, and ART-suppressed macaques. METHODS: RNA was extracted from LN and portions of ileum isolated from pigtailed macaques that were uninfected, infected with RT-SHIV_{mne}, or RT-SHIV_{mne}-infected and suppressed with combination ART for 20 weeks. Nanostring technology was used to digitally measure RNA from 124 inflammation-associated genes, 5 housekeeping genes, and RT-SHIV *gag* from the tissues. Inflammatory genes included cytokines and chemokines and their receptors, integrins, TNF/TNFR superfamily molecules, toll-like receptors, kinases, and transcription factors.

RESULTS: Significant up- or downregulation of many inflammatory molecules was observed in the LN of infected animals compared to controls. The ileum showed differential RNA expression compared to LN and also showed changes after RT-SHIV infection. Similar results were obtained from tissues isolated from uninfected or SIV-infected rhesus macaques. While the expression of many genes returned to normal levels in RT-SHIV-infected macaques after 20 weeks of suppressive ART, some inflammation-associated molecules remained altered in the LN and gut of the treated animals. For example, multiple TNF/TNFR superfamily molecules were reduced after infection compared to the healthy controls and remained low after ART suppression. SIV gag RNA levels were low or undetectable in the uninfected animals and ART-treated animals and significantly higher in the viremic animals. Expression of some inflammatory molecules appeared to correlate with expression of intracellular gag RNA.

CONCLUSIONS: We have identified several specific molecules and pathways that remain abnormal in lymphoid and gastrointestinal tissue in infected animals even after suppressive ART. Additional studies will examine how complex pathways are involved in immune dysfunction, which cell types are affected, and how specific chronic inflammation can be prevented during persistent HIV-1 infection.

ABSTRACT 113

The HIV Viral Protein Nef Downregulates MHC Expression at the Cell Surface Following Reactivation from Latency

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BACKGROUND: A cure for HIV is not currently possible due to the existence of a latent reservoir that is invisible to the immune system and protected from antiretroviral drugs. Upon reactivation, the proviral genome present in this latent reservoir serves as the source for the resumption of viral replication. New therapeutic approaches attempt to reactivate the reservoir to deplete latently infected cells. Although several methods can reactivate the virus, they are unable to purge the latent reservoir, as infected resting CD4+ T cells survived in the presence of cytolytic T lymphocytes. The HIV viral protein Nef downmodulates expression of the major histocompatibility complex, and we hypothesize that this may hide reactivated virus from the host immune response.

METHODS: We established a latency model using both Jurkat T cells and primary CD4+ T cells that were infected by pNL43 HIV harbouring different variations of Nef encoding a dsRed fluorescence marker. Antibodies specific to HLA-ABC (downregulated by Nef) and HLA-E (unaffected by Nef) were used, and data were acquired by flow cytometry and analyzed using FlowJo software.

RESULTS: Infection of Jurkat and primary cells results in the downmodulation of HLA-ABC by the viruses harbouring the different variants of Nef. Infection of Jurkat and primary cells, in the presence of the protease inhibitor, Darunavir, resulted in production of a latent population, detected by dsRed production after reactivation. Upon reactivation, downmodulation of the MHC molecules at the cell surface occurred at the same frequencies as seen with direct infection. Interestingly, infection or reactivation of Nef-deleted viruses induced upregulation of the MHC complex, and viruses containing active Nef brought MHC expression level down to those present in uninfected cells.

CONCLUSIONS: Reactivation of latent virus by current methods may not be adequate to deplete the latent reservoir. Upon reactivation of virus, Nef can downmodulate the MHC at the cell surface to normal levels. This may potentially hide the virus from cytolytic T lymphocytes, resulting in the inability of the immune system to kill infected cells (to be tested) and deplete the viral reservoir. A compound inhibiting this function of Nef may be required for a cure.

ABSTRACT 114

Implication of Interleukin-10 in HIV Persistence

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BACKGROUND: Interleukin-10 (IL-10) has been implicated in the persistence of chronic infections. Blocking IL-10 receptor (IL-10R) signalling restores T cell function and resolves viral infections *in vivo*. We have observed that IL-10 induced by lipopolysaccharide impairs T cell proliferation induced *in vitro* by peptides or mitogen. IL-10 levels also correlate with plasma viral load during HIV infection ; moreover IL-10R, like PD-1, correlates with the frequency of CD4+ T cells harboring integrated HIV DNA. We tested the hypothesis that IL-10 signaling pathway could be implicated in HIV persistence not only by impairing antiviral immunity but also by modulating viral replication and / or survival of infected cells.

METHODS: We developed two models to evaluate the impact of IL-10 on viral production / replication and infected cell survival. In the *ex vivo* model, CD4+ T cells isolated from chronically infected donors, were cultured for 6-9 days in the presence of increasing concentrations of IL-10 with or without CD3/CD28 stimulation. Viral production was measured by ELISA (p24) and cell activation / proliferation by flow cytometry (CD25, HLA-DR, PD-1, Ki67). In the second model, CD4+ cells from uninfected donors were infected *in vitro* with an HIV-GFP virus in order to follow survival of productively infected cells cultured with IL-10 and antiretroviral drugs.

RESULTS: The *ex vivo* model, showed that IL-10 increased spontaneous viral production (2-3 fold) and viral replication induced by CD3/CD28 stimulation as compared to unstimulated or TNFa stimulated cells. This was blocked by antibodies against IL-10R and occurred without affecting the differentiation or proliferation of CD4 T cells unlike other homeostatic cytokines (i.e. IL-7, IL-15). The *in vitro* model showed that IL-10-mediated increase in viral production was associated with the enhanced survival of both infected and uninfected cells.

CONCLUSIONS: These data show that IL-10 could impact on viral persistence by modulating viral replication and cell survival.

ABSTRACT 115

The Effect of Vorinostat on HIVspecific Cytotoxic T Lymphocyte Function

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BACKGROUND: Vorinostat works *in vitro* and *in vivo* to activate HIV transcription in latently infected cells and has been used as the first step in the eradication strategy known as "shock and kill". A second cytotoxic step is then required to kill the virus-producing cells of the reactivated latent reservoir. Primed HIV-specific cytotoxic T lymphocytes (CTL) are capable of killing reactivated cells. Vorinostat, licensed for the treatment of T-cell lymphoma, works by inducing a variety of other negative effects on T cells. Recent reports indicate Vorinostat may decrease interferon-gamma secretion by CTL and may block T cell receptor signaling in HIV and SIV infection, respectively. Therefore, we sought to determine the effect of Vorinostat on HIV-specific CTL function *in vitro*. METHODS: Intracellular cytokine levels and markers of degranulation were measured for both bulk CD8+ cells and HIV-specific CD8+ CTL clones 24, 48 and 96 hours after a 48-hour incubation with 0, 30, or 350nM of Vorinostat. Infected cells were co-cultured with HIV-specific clones SL9(Gag) or IV9(RT) that had been pre-incubated in media containing Vorinostat as above. Viral suppression was measured by comparing the increase in p24 between co-cultures with and without CTL.

RESULTS: Levels of IFN-gamma, TNF-alpha, CD107a, and Perforin were decreased in cells treated with 30nM or 350nM Vorinostat compared to untreated controls. The effect was greatest 24-48 hours post-treatment. Levels of IL-2 were low in all cells, treated or untreated. There was no inhibition of CTL-mediated suppression of viral replication. In fact, there appeared to be a trend for increased inhibition of HIV replication with Vorinostat-treated CTL clones.

CONCLUSIONS: Although Vorinostat does appear to decrease cytokine secretion and markers of degranulation, this does not appear to correlation with any negative affect on the ability of HIV-specific CTL clones to kill infected cells in an *in vitro* co-culture assay. The use of a single dose of Vorinostat *in vivo* is unlikely to result in significant inhibition of HIVspecific CTL activity. However, further study is required to determine the effect of repeated frequent doses, which may result in more significant inhibition of CTL function.

ABSTRACT 116

Circulating T Follicular Helper Cells are Preferentially Infected by HIV and are a Potential Cellular Compartment for HIV Reservoir

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OBJECTIVES: Lymph node T follicular helper (Tfh) cells constitute a major CD4 T cell compartment for HIV-1/SIV infection and replication. Here we investigated whether the CD4 T_{CM} subset expressing the chemokine receptor CXCR5, termed peripheral (p)Tfh cells, shared this permissiveness of HIV.

METHODS: We investigated total cellular HIV-1 DNA by real time PCR in purified pTfh and inducible HIV p24 by flow cytometry in CD8 depleted PBMC derived from three HIV-1 infected aviremic patients on >48 wks of potent combination antiretroviral therapy (cART). Permissiveness of pTfh to infection with *NL4.3-BaL-GFP* was evaluated in cells from five healthy HIV negative donors.

RESULTS: In chronically HIV infected patients total cellular HIV DNA was similar in pTfh and non-pTfh cells (1687±635 and 1864±564 copies/million cells respectively) following 48 wks of cART. Frequencies of inducible HIV p24 gag expression, following antiCD3+CD28 treatment for 72 hrs in presence of raltegravir, showed similar levels in $T_{\scriptscriptstyle CM}$ (3.7±1.8%) and T_{TM} (3.8±2.1%) cells. However, when T_{CM} were further gated on pTfh and non-pTfh cells, p24 expression was significantly higher (p=0.019) in pTfh cells (14.2±4.6%) compared to non-pTfh cells (2.2±1.1%). In cells from healthy HIV negative donors the in vitro infection assay in purified memory CD4 T cell subsets showed pTfh cells to be highly permissive to infection (GFP+) compared to non-pTfh cells and PD-1+pTfh were even more permissive, either without or with prior PHA activation. Although expression of activation markers (HLA-DR, CD38), Ki67, CCR5 and PD1 levels were similar between pTfh and non-pTfh cells, the frequency of PD1 expressing cells was significantly higher in pTfh cells than non-pTfh cells (p=0.038) after PHA stimulation.

CONCLUSION: HIV-1 infected aviremic patients on >48 wks of potent cART harbor inducible HIV as indicated by the expression of gag p24 which was maximal in pTfh cells expressing PD-1. Cells designated as pTfh cells in the peripheral blood are permissive to HIV infection with and without prior activation. pTfh in peripheral blood could constitute a major HIV reservoir that could serve as a biomarker for monitoring HIV reservoirs.

ABSTRACT 117

Profound Alterations in Cholesterol Metabolism Restrict HIV-1 *Trans* Infection of CD4 T Cells in Viremic Controllers

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BACKGROUND: HIV-1 controllers inhibit disease progression without antiretroviral therapy. We assessed whether the ability of viremic controllers (VC) to control disease progression was related to the ability of dendritic cells (DC) and B cells (professional APC) from VC to mediate HIV-1 *trans* infection of T cells, and its association with cholesterol metabolism.

METHODS: HIV-1 *trans* infection of autologous CD4 T cells by APC was tested for 8 VC, 8 progressors (PR) and 8 seronegatives (SN) in the Multicenter AIDS Cohort Study. Total cell cholesterol content and efflux was measured, as well as cellular levels of the cholesterol 1transporter ABCA1. APC from VC were treated with siRNA to inhibit ABCA1-mediated cholesterol transport, and from SN with nuclear receptor ligands to upregulate cholesterol efflux and Levastatin to inhibit cholesterol synthesis.

RESULTS: Strikingly, APC from 0/8 VC *trans* infected CD4 T cells with HIV-1, whereas APC from 8/8 PR and 7/7 SN demonstrated efficient *trans* infection. There was no difference

in direct (*cis*) HIV-1 infection of T cells from the 3 groups. Importantly, APC from VC had impaired *trans* infection both prior to and after primary HIV-1 infection, whereas APC from PR mediated *trans* infection both before and after seroconversion. APC but not T cells from VC showed significantly lower cholesterol content and higher efflux compared to SN and PR. Statin treatment reduced *trans* infection by APC from SN, while interference with the ABCA1 pathways in VC restored their ability to transmit virus. Levels of the phosphorylated form of ABCA were higher in APC from VC compared to PR. Modified mRNA transcripts related to cholesterol metabolism were identified in APC from VC compared to PR.

CONCLUSIONS: We show that APC from VC lack the ability to *trans* infect T cells with HIV-1. This was associated with profoundly enhanced cholesterol metabolism that appears to be a genetic trait. Our results provide important new clues to control of HIV-1 infection.

ABSTRACT 118

An *ex vivo* Model to Evaluate Therapies that Target Reservoirs

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BACKGROUND: Several strategies have been proposed to target the latent reservoir for expression and clearance in patients on suppressive ART. Thus, there is a need for physiologically relevant models to test the effect of candidate agents on reservoir expression and clearance. All prior models, to our knowledge, utilize superinfection. In one such superinfection model we demonstrated that monitoring integrated HIV DNA was a useful surrogate for reservoir clearance. Herein, we describe an ex vivo model that does not require superinfection but rather monitors integration as a surrogate of reservoir clearance. We show the utility of this model by evaluating two therapeutic agents on reservoir size. METHODS: We developed an ex vivo co-culture system using CD4 and CD8 T-cells purified from PBMCs of individuals on HAART with undetectable viral loads. CD8s were expanded by exposing PBMCs to Gag peptides for 5 days before purification. CD4 T-cells were purified from a separate vial of frozen cells from the same patient and cultured for 2 days. CD4s and CD8s were then co-cultured for 2 days. We evaluated reservoir clearance following addition of two stimuli. Integrated HIV DNA was measured by Alu-Gag PCR before and after co-culture and normalized to the number of CD4 T-cells present in the culture.

RESULTS: We demonstrate that our ex vivo model can capture clearance of integrated HIV DNA from patient CD4 T-cells in co-culture when reservoir expression was induced by two different stimuli. Two out of 3 patients showed a decrease in integrated HIV levels following stimulation with either interferon- α or SAHA. We also found that a patient with this responder phenotype showed similar decreases in integration after co-culture when cells were isolated from a sample obtained several years earlier, suggesting that responsiveness is an inherent quality of the reservoir. Importantly, results from this ex vivo system paralleled results obtained in a recent trial of *in vivo* interferon- α treatment, suggesting that this model may be useful for predicting clinical effectiveness.

CONCLUSION: An ex vivo co-culture model may be useful for evaluating therapies and probing the mechanism of agents that target the latent reservoir.

ABSTRACT 119

Irradiation-induced Cellular Stress Activates Virus Replication and Apoptosis of Hiv-1 Infected Cells *in vitro* and *in vivo*

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BACKGROUND: The highly active antiretroviral therapy reduces HIV RNA in plasma to undetectable level. However, the virus continues persistence in resting T cells and other reservoirs, such as T-cells, perivascular macrophages, microglia and astrocytes. Selective reactivation and eradication of HIV from various reservoirs is a critical problem of current HIV therapy. The X-ray irradiation (IR), well-defined stress signal, that is widely used for many therapeutic purposes, has earlier been shown to be capable to activate increased HIV-1 transcription, progeny virion formation and eventual apoptosis of infected cells.

METHODS: A standard x-ray IR instrument was used to irradiate cells as well as whole animals. Doses were calculated based on intensity and time of exposure. Samples were either left at 37°C after IR or animals were put back into their cages for up to a month.

RESULTS: Here, using the HIV-1 infected T-cells and monocyte-derived macrophage (MDM) model cells and NSG humanized mice infected with dual-tropic HIV-1 89.6 strain, we examined the effect of IR-induced cellular stress on HIV-1 replication and viability of infected cells. Treatment of both T-cells and MDM with different IR doses led to dramatic increase of HIV-1 transcription, as evidenced by presence of Pol II and reduction of HDAC1 on HIV-1 promoter when using ChIP assay. Incubation of IR-treated cells with proteasomal inhibitor resulted in the additional increase of HIV-1 transcription by stabilizing Tat protein. Interestingly, analysis of infectivity of progeny virions using TZM-bl reporter cells showed decreased infectivity of the virus produced by irradiated cells, suggesting that along with activation of HIV-1 replication, IR increased production of defective viral particles. Treatment of HIV-1 infected humanized mice that did not display viral RNA in the plasma with IR resulted in significant increase of HIV-1 RNA in plasma, lung and brain.

CONCLUSIONS: Taken together, our current data suggest that IR-induced cellular stress activates HIV-1 expression in the infected T-cells, and MDM-rich tissues and facilitates the apoptotic death of infected cells possibly via Tat-dependent phosphorylation of p53 protein. The feasibility and use of IR to activate the virus and its possible mechanisms in both T-cells and MDMs will be discussed.

ABSTRACT 120

Well-mixed Virus Populations Across Tissue and Blood of RT-SHIV-infected Macaques on Suppressive cART Argues Against Foci of Viral Replication in Pharmacologic Sanctuaries

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BACKGROUND: Determining which compartments contribute to plasma HIV is critical to understanding the sources of viremia during cART. We analyzed RT-SHIV populations in plasma and tissues from infected macaques before and during treatment with cART to assess the sources and mechanisms of residual viremia.

METHODS: Tissues were collected at necropsy from pigtailed macaques infected for 30 weeks with a diverse population

of RT-SHIV_{mne}. Two of four animals were treated with EFV+TDF+FTC for twenty weeks. A total of 1800 singlegenome HIV *pol* and SIV *env* DNA and RNA sequences were analyzed from the plasma, PBMCs, lymph nodes, spleen, thymus, gut tissues, bone marrow, and lung. Populations in each tissue were compared to plasma virus using phylogenetic and panmixia tests.

RESULTS: Viral DNA and RNA levels in one untreated animal were substantially lower than the other (inter-tissue mean 77 DNA copies/106 cells in animal 8232 vs. 265 DNA copies/10⁶ cells in animal 6760; and $\sim 10^2$ vs. $\sim 10^5$ plasma RNA copies/ml, respectively). Plasma RNA levels in the two treated animals were undetectable and the mean viral intertissue DNA levels were 41 and 113/106 cells (8030 and 8272, respectively). Phylogenetics of intracellular RNA populations revealed that the majority of proviruses in tissues from 8232 were not expressed, whereas tissues from 6760 had a greater proportion of proviruses being expressed. Few intracellular RNA sequences were detected in treated animals and most contained frame shifts or large deletions. HIV pol and env populations in tissues were diverse but were not distinct from virus populations in plasma in either treated or untreated animals. By contrast to the overall env sequences, the V3 regions alone exhibited very little diversity, consistent with purifying selection on this region.

CONCLUSIONS: These findings indicate that levels of plasma viremia in untreated macaques are related to both the number of infected cells and to the proportion that express RNA, that virus populations in plasma and tissues are well mixed (arguing against virus replicating in tissue-specific pharmacologic "sanctuaries" during cART), and that strong purifying selection of V3 can misrepresent diversity of viral populations unless conserved regions of envelope are also examined.

ABSTRACT 121

Transcriptional Regulation and Pharmacologic Reactivation of Latently Infected CD4 T Cells Isolated from FIV-infected Cats

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BACKGROUND: Feline immunodeficiency virus (FIV) infection of cats is an important animal model for HIV-1 pathogenesis and is responsible for feline AIDS. FIV is phylogenetically related to HIV and is capable of infecting CD4 T cells as well as monocytes, astrocytes and CNS microglia. Our studies have documented that FIV establishes a latent infection in peripheral blood CD4 T cells.

METHODS: Specific pathogen free cats were experimentally infected with the feline immunodeficiency virus (FIV)-C strain and allowed to progress to the asymptomatic phase of infection, approximately 10 months post infection (PI). CD4 T cells were isolated by immunomagnetic methods and the cellular viral load determined through serial dilution and real-time PCR assays. The local chromatin status was discerned through chromatin immunoprecipitation (ChIP) assays. *Ex vivo* pharmacologic reactivation of CD4 T cell viral latency was assessed by ChIP, real-time PCR and supernatant reverse transcriptase activity.

RESULTS:L FIV-C infected cats in the asymptomatic phase demonstrated an infectious viral load of approximately 1 infected cell per 10⁵ CD4 T-cells. The latent FIV proviral promoter was associated with deacetylated, methylated histones, consistent with a condensed chromatin structure. Lately infected CD4 T cells were readily reactivatable with histone deacetylase inhibitors and a histone methyltransferase inhibitor. All compounds tested were able to alter histone lysine residue modifications in feline cells, both globally and at the FIV promoter locally. When latently FIV-infected peripheral CD4+ T-cells were cultured *ex vivo* in the presence

of these inhibitors, viral transcription was significantly activated relative to no treatment controls.

CONCLUSION: Lentiviral latency within *in vivo* derived FIV-infected CD4 T cells is epigenetically mediated through a restrictive chromatin environment. Our findings for the FIV promoter in peripheral blood of infected cats are similar to results reported for HIV-infected CD4 T cells. The FIV/cat model may offer mechanistic insights into *in vivo* mechanisms of HIV latency and provides a unique opportunity to test novel therapeutic interventions aimed at eradicating latent virus.

ABSTRACT 122

M1 Polarization of Primary Human Macrophages Leads to Restriction of HIV-1 Replication by Affecting Multiple Steps of the Virus Life Cycle

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BACKGROUND: Mononuclear phagocytes represent a relevant target of HIV-1 infection displaying both common and distinctive features in comparison to CD4+ T cells. Among these, a higher resistance to the cytopathic effect caused by the infection and a propensity to accumulate virions in intracellular compartments. Macrophage activation by multiple cytokines has been previously shown to significantly affect virus replication. This process has been recently revisited according to an "M1/M2 paradigm" of functional polarization similar to the Th1/Th2/Th17 polarization of T lymphocyte responses. We have, therefore, earlier investigated the potential role of M1/M2 polarization on the susceptibility of human primary monocyte-derived macrophages (MDM) to HIV-1 infection and observed a potent restriction of virus replication particularly in M1-MDM that was further investigated.

METHODS: Human MDM established from seronegative donors were stimulated for 18 h with IFN-[] and TNF-[] to induce the M1 phenotype; cytokines were then removed and both M1-polarized and control MDM were infected with either R5 HIV-1 or VSV-g pseudotyped viruses, used in order to bypass receptor/coreceptor entry requirement. HIV-1 virion production was measured in cell culture supernatants by Mg2+-dependent RT activity assay. The levels of viral transcripts and of HIV DNA (both total and integrated) were quantified by Taqman assays. The expression of candidate HIV restriction factors (RF), including APOBEC 3G (A3G), A3A, TRIM22 were quantified by Taqman and/or by Western blotting.

RESULTS: M1-MDM were profoundly restricted in their capacity to support the replication of both R5 HIV-1 and of VSV-g pseudotyped viruses. A significant reduction of total HIV-1 DNA synthesis was associated with a delayed integration of proviral DNA and a reduction of virus transcription. When candidate RF were explored for their expression in control and M1-MDM, only A3A, but not A3G, emerged as differentially induced in M1-MDM, suggesting its potential role in the observed phenotype of restricted virus replication.

CONCLUSIONS: M1 polarization of macrophage activation leads to a profound restriction of virus replication in these cells suggesting its potential role as a defense mechanism during infection. The potential role of A3A in the restriction of virus replication in M1-MDM is currently pursued.

ABSTRACT 123

High Levels of CD2 Expression Identify HIV-1 Latently Infected Resting Memory CD4⁺ T Cells in Virally Suppressed Subjects

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BACKGROUND: Resting memory CD4⁺ T cells harbor dormant, stably integrated HIV-1 for years even in the context of suppressive antiretroviral therapy, representing a major obstacle to a cure. Current eradication strategies involve viral reactivation in the absence of global T cell activation. However, there is a need to develop additional strategies, such as direct elimination of latently infected cells through tagging for clearance by the immune system. The lack of surface markers identifying latently infected cells has so far hindered the development of such approaches.

METHODS: We established a culture system to generate latently infected and uninfected CD4⁺ T cells *in vitro*. We used this model to identify surface markers differentially expressed in these two cell subsets. We confirmed the results by RT-QPCR and flow cytometry. Differential expression of the cell surface marker CD2 on latently infected cells *in vivo* was validated by sorting resting memory CD4⁺ T cells expressing high and low surface levels of CD2 from PBMC of six HIV-1 subjects successfully treated with antiretroviral drugs for at least three years. Then, we used ultrasensitive PCR techniques to compare total HIV-1 DNA copies and viral RNA produced following *ex vivo* reactivation from the two cell subsets.

RESULTS: Microarray analyses revealed profound differences in the gene expression profiles of latently infected and uninfected cells generated *in vitro*. We found that 33 differentially expressed transcripts encoded for CD surface markers. Some of them were validated by RT-QPCR and flow cytometry, which confirmed higher cell surface expression of CD2 on latently infected cells *in vitro*. We also found that resting memory CD4⁺CD2^{high} T cells from 6 out of 6 virally suppressed HIV-1 positive subjects harbored higher HIV-1 DNA copy numbers than total CD4⁺ T cells (median 5.7-fold, p = 0.013, paired *t*-test). After *ex vivo* viral reactivation, robust viral RNA production was detected only from resting memory CD4⁺CD2^{high} T cells, but little or no viral RNA was produced from other cell subsets.

CONCLUSIONS: These results indicate that high expression of the surface receptor CD2 identifies resting memory CD4⁺ T cells harboring replication competent HIV-1 in peripheral blood of virally suppressed subjects.

ABSTRACT 124

Can HIV go into Latency in Activated and Proliferating CD4 T Cells Under Strong HIV-specific Cytotoxic T Cell Pressure?

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BACKGROUND: The mechanism of how HIV latency is established in resting memory CD4 T cells in patients is still not clear. Although several primary T cell models are currently available to study HIV's reactivation from latency, it remains uncertain if any of these models lacking HIV-specific cytotoxic T cell (HIVspCTL) pressure actually mimic the formation of latently infected CD4 T cells present *in vivo*. Conceivably, developing a primary T cell model with built-in HIVspCTL pressure would be important to study the mechanism of HIV latency *in vitro*. First, we sought to examine if, at all, HIV can go into latency in activated and proliferating CD4 T cells *in vitro* under a strong HIVspCTL pressure.

METHODS: The gene-modified CD8 T cells with either chimeric CD4-CD3ζ or CD4-CD28-CD3ζ receptors (CD4-CARs) are referred here as HIVspCTLs, because of their profound ability to target and kill productively infected cells in vitro. Normal donor's blood derived CD45RA+CD4+ T cells were stimulated with anti-CD3/CD28 beads, followed by 10-12 days of robust cell-expansion in media containing 300U/ml IL-2. The activated and proliferating CD45RO+CD4+ T cells infected overnight with a R5-tropic virus, HIV_{IRCSP}? were cocultured with HIVspCTLs at 1:1 ratio for 6 days. To measure the kinetics of HIV replication, culture supernatants were tested periodically for the levels of HIV-p24 by ELISA. Following 6 days of treatment with HIV inhibitors, CD4 cells were purified from cocultures and subjected to virus outgrowth assays for detecting latent but inducible HIVs, if established, in these cells.

RESULTS: As expected, there were substantially limited levels of HIV replication in activated and proliferating CD4 T cells when cocultured with HIVspCTLs. However, interestingly, a small fraction of CD4 T cells recovered from cocultures possessed latent HIVs that could be reactivated to productive phase with various T cell stimuli. These data suggested that absent to limited levels of viral gene expression in some of the fully activated and infected CD4 T cells likely concealed these cells from HIVspCTL-mediated elimination, resulting in the establishment of HIV latency.

CONCLUSION: HIV can go into latency in activated CD4 T cells *in vitro* with limited viral gene expression, effectively escaping HIV-specific CTLs present in cocultures. This observation might reflect a potential new mechanism of how latent HIVs are formed in resting memory CD4 T cells *in vivo*.

ABSTRACT 125

Study of Gene Expression and Latency Properties in Different HIV-1 U3 Subtypes

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BACKGROUND: So far, countless studies have explored the mechanisms of HIV-1 latent infections. However these studies have mainly focused on HIV-1 subtype B, despite the extensive sequence diversity in the 5' long terminal repeat (LTR) among different HIV-1 subtypes. HIV-1 gene expression is largely dependent on inducible host transcription factors that interact with numerous cis-regulatory elements within the U3 region of the 5'-LTR. Using LTR-GFP-IRES-Tat (LGIT) lentivirus with unique U3 subtype isolates in Jurkat and primary CD4+ T cells, we recently demonstrated that variability of Sp1 and NF- κ B binding site in U3 region among subtypes have a major impact on emergence of latency. In addition, these latent subtypes were efficiently reactivated by treatment with the combination of anti-latency drugs, prostratin and SAHA. In this study, we employed a primary CD4+ T cell latency model using replication-competent HIV-1 NL4-3-based fulllength virus (sLTR) containing subtype-specific U3 regions in order to see their replication dynamics as well as to test for reactivation using various anti-latency drugs.

METHODS: Primary CD4+ T cells isolated were activated and infected with the sLTR virus containing various U3 subtype isolates. Aliquots of the culture supernatant were collected, then analyzed using p24 ELISA. Meanwhile, the infected cells were treated with the integrase inhibitor. After 10 days, the cells were activated with anti-latency drugs, (e.g. prostratin and SAHA), either individually or in combination. The reactivation was analyzed by intracellular p24 staining, flow cytometry.

RESULTS: Over the time course assay, we observed striking differences in replication dynamics among U3 subtypes, which suggested that there might be differences in latency establishment as well as susceptibility to anti-latency drugs. Prostratin and SAHA each reactivated latency at modest levels, and the degree of reactivation varied among subtypes. Importantly, the combination of these drugs showed synergistic reactivation.

CONCLUSIONS: Using replication-competent virus, we found that different U3 subtypes exhibit different replication rates, which imply contribution of the architecture of U3 regions to latency establishment. Also, as with our previous observation, the combination of prostratin and SAHA synergistically reactivated latency in CD4+ T cells. These results may contribute to developing new approaches for HIV-1 latency reactivation.

ABSTRACT 126

Targeted Nucleases to Create HIV Resistance

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Targeted nucleases such as zinc finger nucleases (ZFNs), TALENs and CRIPSR/Cas systems can be engineered to create double-stranded breaks at a specific DNA target, with the possible outcomes being gene knockout, gene editing, or gene addition. For HIV therapies, the technology is being applied to disrupt the CCR5 co-receptor gene, promote site-specific
addition of anti-HIV genes, or create HIV-specific nucleases that could be used to disrupt any integrated proviruses in reservoir cells. Disruption of CCR5 by ZFNs is the most clinically advanced application and is being used to engineer ex vivo expanded T cells from HIV-infected individuals to provide HV-resistance. Ongoing work in our group is directed towards a proposed clinical trial based on hematopoietic stem cell (HSC) engineering with the same ZFNs. Towards this goal, we have developed highly efficient gene disruption techniques based on mRNA electroporation that work with both ZFNs and TALENs, and results in disruption rates of 30-70% of the CCR5 alleles in human HSC populations. Importantly, these treatments have no impact on cell viability or function as assessed by transplantation into humanized mice, and give rise to CCR5-negative T cell progeny that can suppress HIV replication in the same animal model. The use of mRNA to deliver targeted nucleases is an attractive and potentially safer alternative to classical viral vectors, obviating the potential immunogenicity of viral components and reducing any potential risk of insertional mutagenesis, while enabling permanent and targeted genetic modification of the HSC following only a transient exposure to the ZFNs. Electroporation of mRNA thus provides an efficient delivery system for ZFN-driven CCR5 disruption in HSC that can be readily exploited for the clinical scale manufacture of CCR5disrupted HSC for human trials.

ABSTRACT 127

Human Methyltransferase Inhibitors for Purging HIV-1 from the Latent Reservoir

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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 repeat (LTR). 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 histone methyltransferase inhibitors (HMTIs) namely Chaetocin and BIX-01294 in reactivating HIV-1 from latency.

METHODS: We used CD8⁺ T-cells depleted peripheral blood mononuclear cells (PBMCs) isolated from 13 HIV⁺ HAART-treated patients with undetectable viral load for a period over 4 years. We measured HIV-1 recovery in exvivo 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 a control samples.

RESULTS: Chaetocin and BIX-01294, induced recovery of HIV-1 virions from CD8+-depleted cell cultures of 10 out of 13 subjects. Second day after treatment with the drugs, culture supernatants were tested using bDNA of viral load assay and the results revealed HIV-one mergence from 03 day to 29 day (median of 09 days) and viral loads from 2.2 log10 to 6.0 log10 (median of 5.7).

CONCLUSION: We showed here that these nonadministrable HMTIs may provide a therapy to purge latent HIV-1 from reservoirs possibly in combination with other chromatin remodeling drugs. Therefore, clinical grade HMTIs should be synthesized and evaluated for their reactivation potential from the latent reservoirs that are the biggest hurdle towards the sterilizing HIV cure.

ABSTRACT 128

Natural Killer Cells for Clearing the Reactivated Latent HIV Reservoir

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BACKGROUND: Efforts to eradicate HIV infection have been directed towards reactivation of latent proviruses. However, it has been suggested that reactivation will not clear the viral reservoir. Enhancing the host immune system may improve its ability to eliminate reactivated infected cells. We studied the ability of cytokine-stimulated Natural Killer (NK) cells to clear HIV after reactivation.

METHODS: PBMCs from HIV+ patients were obtained by leukopheresis. CD4+ T-cells and NK cells (CD56+) were negatively selected. NK cells were stimulated for 24-hours with 100U IL-2/mL. NK-mediated-cytotoxicity was evaluated by a) measurement of percent inhibition in a viral inhibition assay b) measurement of the degranulation marker CD107a by flow cytometry, and c) using a novel latency clearance assay based in an outgrowth culture where resting CD4+cells are reactivated and cultured with or without effectors in replicate wells.

RESULTS: Viral inhibition was performed on cells of five patients: three were superinfected with JR-CSF and two with autologous reservoir virus obtained from a past outgrowth assay. Unstimulated NKs reduced virus production an average of 74.53%(p=0.02), 30%(p=0.03) and 6%(p=0.680) at effector:target ratios of 1:1, 1:10 and 1:100, respectively. Stimulated NKs reduced viral production 87.3%(p=0.000), 37.87%(p=0.009) and 15.55%(p=0.354) at 1:1, 1:10 and 1:100 ratios. Cytotoxicity assays were performed in three patients. NK cells were incubated alone and with autologous non-superinfected or superinfected CD4+T-cells. Average %CD56+cells expressing CD107a was 3.219(SE±=0.123) and 12.522(SE±4.631) for NK and stimulated-NKs incubated alone; 3.987(SE±0.672) and 13.264(SE±4.996) respectively when NKs were incubated with non-superinfected cells, and 6.614(SE±1.132) and 16.544(SE±4.945) when incubated with superinfected cells. In nine latency clearance assays, a mean of 43.98% (SE±7.58) of wells without effectors produced virus, compared to 28.70%(SE±7.49) of wells cultured with NKs (p=0.217). IL-2 stimulated NKs were used in two experiments, resulting in an average of 17.15% of positive wells.

CONCLUSIONS: NK cells showed a strong antiviral effect in the autologous viral inhibition assay, and a modest but positive effect in clearing HIV infected cells upon reactivation in the outgrowth assay. IL-2 stimulation resulted in improved NK performance in all experiments, suggesting that stimulating NKs with cytokines may enhance their function in immune interventions used along with anti-latency therapies.

ABSTRACT 129

Effect of Multi-dose Vorinostat on Host Gene Expression in HIV Infected Patients on Antiretroviral Therapy

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BACKGROUND: Histone deacetylase inhibitors (HDACi) activate HIV production in latently infected T-cells *in vitro*. Recently, the HDACi vorinostat was shown to also significantly increase cell associated unspliced (CA-US) HIV RNA in in HIV infected subjects on suppressive antiretroviral therapy (ART). However, the effects of vorinostat on host gene regulation and the relationship of these changes to efficacy in activation of HIV transcription is unknown. The main aim of this study was to determine the effects of multi-dose vorinostat on host gene expression in HIV-infected patients on ART.

METHODS: We evaluated differential gene expression in blood from 20 HIV-infected patients on ART who received vorinostat (400mg/day) for 14 days. Gene expression was analyzed at baseline and two time points on vorinostat (d1 and d14) and post-cessation of vorinostat (d84). Differential gene expression was assessed using a (moderated) *t* test. For data mining and functional analyses, the genes selected satisfied a false discovery rate (FDR) corrected p-value \leq 0.05.

RESULTS: There was clear difference in gene expression for all patients at d1, d14 and d84 compared to baseline. At d1, there was a distinct pattern of response in 7/20 patients with marked up-regulation of genes and enrichment of pathways involved in transcription, chromatin remodeling, and translation elongation. Transcriptional activity associated with chromatin remodeling disappeared by day 14 in all patients but there was persisting differential gene expression at day 14 and day

84 compared to baseline. At day 14, pathways associated with reduction of oxidative stress/inflammation and genes associated with DNA repair were differentially expressed. At day 84, the major differentially expressed genes were alpha defensins. Regression analysis of gene expression and CA-US HIV RNA revealed distinct transcriptomic profiles correlating with high and low levels of CA-US HIV RNA at day 1.

CONCLUSION: These results suggest that the effect of vorinostat on chromatin largely occur within the 1st day after the first dose of drug and that after 14 days of continuous dosing, there are compensatory mechanisms associated with transcriptional repression and cell survival.

ABSTRACT 130

Mathematical Modeling of HIV-1 Latent Reservoir Dynamics Following Hematopoietic Stem Cell Transplantation

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BACKGROUND: Allogenic hematopoietic stem cell transplantation (HSCT) is capable of reducing the latent reservoir (LR) in HIV-1-infected patients and delaying viral rebound following interruption of antiretroviral therapy (ART). However, little is known about how many infected cells may remain after transplant and how long patients should be followed after treatment interruption.

METHODS: We used previously reported data collected from two patients who underwent allogeneic HSCT from CCR5 wild-type donors. Due to lack of detectable HIV-1 DNA and RNA up to 4.3 years post-HSCT, both patients interrupted ART and remained rebound-free up to the last reported sample time-points of 8-15 weeks. Available data included longitudinal plasma RNA levels, microchimerism, HIV-1 DNA and co-culture from CD4+ T cells. We used a mathematical modeling framework to estimate the residual LR in host cells and the number of potential newly infected donor cells present at treatment interruption. We derived a distribution for the time of viral rebound, and the probability that the reservoir is completely cleared, as a function of the remaining infected cells. We present a method to update LR size estimates in real-time as patients continue off treatment, and to estimate the time after which rebound becomes unlikely.

RESULTS: Residual latent reservoirs may be present, but at levels orders of magnitude below the limit of detection of experimental assays necessitating other methods to estimate viral rebound probabilities and times. At week 8 and 15 off therapy, there is high probability that the patients' total reservoir sizes are less than 15,000 and 6000 cells, respectively. If patients experience rebound after 3 or 6 months after treatment interruption, the best estimates for the remaining size of the reservoir would be 1600 and 800. With no prior estimate for the remaining size of the reservoir other than the assay detection limit, many years of follow-up would be needed to ensure the probability of future rebound is small.

CONCLUSIONS: Mathematical models and parameters of viral dynamics can be used to provide guidance for treatment interruptions following HSCT. These models are important when experimental estimates are limited by the dynamic range of current assays for residual infection.

ABSTRACT 131

Long-term Follow-up of a Primary HIV Infection (PHI) Cohort with Over 4 Years of ART

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BACKGROUND: Eradication strategies are now being developed with the hope of achieving either functional cure (FC) or eradication (ER). Viral reservoirs (VR) of integrated HIV-1 remain one of the main obstacles to achieve such a goal. We and other have shown low levels of VR as measured by cell-associated DNA in treated PHI (Plos One 2011 Cellerai et al). Very prolonged ART initiated at PHI may theoretically be associated with one of the best opportunities for ER/FC. Little data is available on PHI cohorts with ART>4 years and whether patients can be maintained on long-term ART initiated during PHI.

METHODS: Review of medical files of early PHI subjects: (1) <<u>3</u> bands WB/low avidity test and 2) ART initiation \leq 3 months post diagnosis) referred to our seroconversion clinic for treatment advice with follow-up (FU) on ART for >4 years. Subjects characteristics, ART duration and rate of discontinuation after 4 years of ART, HIV-1 viral load (VL) and CD4 at last follow-up, type of ART received.

RESULTS: 38 subjects fulfilled the inclusion criteria (male/ females 36/2), of which 36 were under FU after having completed >4 y of ART. Mean age at last FU (n=38); 46 y (29-69 y). Mean ART duration was 111.7 months (51-151). 37/38 subjects initiated ART with PI. Blips >50 HIV-1 copies/ mL occurred in 2/38 subjects. Both subjects later resupressed due low adherence level. Two subjects decided to discontinue ART after >4 years and are under FU.

CONCLUSION: low level of treatment discontinuation in subjects having completed >4 years of ART initiated at seroconversion. Long-term VL control (<50 HIV-1 c/mL) on ART was achieved in most subjects. Assessment of VR using newly developed virological assays (target capture and deep sequencing) is planned in this cohort. We believe that such cohorts should be considered for future eradication strategies.

ABSTRACT 132

Novel CD4-based Chimeric Antigen Receptors as Immunotherapy for an HIV Functional Cure

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BACKGROUND: Chimeric antigen receptors represent a promising strategy for functional cure of HIV. We engineered three CARs containing identical intracellular signaling domains (CD28 + CD3 ζ) linked to extracellular derivatives of CD4 (D1D2). These included CD4 alone (CD4 CAR) or attached to the 17b scFv targeting the highly conserved coreceptor binding domain, using either a long (35 aa; CD4-35-17b) or short (10 aa, CD4-10-17b) linker. Previous studies indicated that the corresponding soluble bifunctional proteins containing a long linker (sCD4-35-17b) neutralized HIV-1 strains with extremely high potency and breadth due to simultaneous binding of both sCD4 and 17b moieties to the same gp120 subunit; a protein with a linker too short for simultaneous binding (as in sCD4-10-17b) displayed only modest potency, comparable to sCD4 alone. Based on alternative concepts regarding the influence of CAR binding affinity, we sought to compare the relative functional activities of these 3 CARs.

METHODS: T cells were transduced with the CAR constructs and tested for anti-HIV activity using various *ex vivo* assays.

RESULTS: CD8 T-cells modified by each CAR were functionally activated upon Env engagement to secrete IFN- γ secretion, kill Env⁺ targets, and block HIV production by activated CD4 cells from infected subjects. All three CARs effectively suppressed HIV-1 infection of PBMC; the sCD4-10-17b CAR was significantly more potent than the CD4 CAR, and much more potent than the sCD4-35-17b CAR. These results support a model whereby T cell killing efficacy is compromised when the affinity between effector and target cell molecules is too high to permit "serial triggering". Strikingly, the CD4 CAR rendered CCR5⁺ cells highly susceptible to HIV-1 pseudovirus infection, an undesireable effect not observed with the bifunctional CARs due to both coreceptor binding site blockade and steric hindrance by the 17b scFV.

CONCLUSIONS: The novel CD4-10-17b CAR design offers both superior potency and safety.

ABSTRACT 133

Novel Activators and Nanoparticle Delivery Methods for Eliminating Latent Reservoirs of HIV

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AIMS: Latently-infected CD4+ T cells represent a key barrier preventing the cure of HIV infection in patients treated with antiretroviral therapy (ART). One approach for eliminating this latent reservoir is to induce the virus to express new proteins, which would make the host cell susceptible to viral cytopathic effects, immune effector mechanisms, and other therapeutic approaches targeting viral proteins. For this strategy to be successful, safe and effective methods for activating latent HIV expression are needed. Our goals were to design and synthesize novel latency activating compounds that function via the protein kinase C (PKC) signaling pathway, and to produce new nanoparticles that are capable of more selectively introducing latency activating compounds into the cell type of interest.

<u>METHODS</u>: Synthetic analogs of the natural PKC-activating compounds prostratin and bryostatin were designed and synthesized. These were evaluated in cell line models for HIV latency, several primary cell assays, and latently-infected cells obtained ex vivo from ART-treated patients. To enhance delivery of latency-activating compounds, new nanoparticles based on naturally occurring "vault" particles were produced and evaluated. These vault nanoparticles were engineered to incorporate an amphipathic helix allowing binding and packaging of hydrophobic PKC activators, and targeting with CD4-specific antibodies.

RESULTS: Novel prostratin analogs were capable of potently activating HIV from latency at concentrations over 100-fold lower than the natural product. Several bryostatin analogs also activated HIV from latency more effectively than the natural product. Vault nanoparticles loaded with bryostatin 1 were capable of activating HIV from latency, and were bioactive (induced CD69 expression) in CD4+ splenocytes following intravenous administration to immunocompetent mice.

CONCLUSIONS: Novel PKC activators and nanoparticle delivery methods may prove useful in activation-elimination approaches intended to purge the latent reservoir of HIV.

ABSTRACT 134

A Pilot Study Assessing the Safety and Latency Reversing Activity of Disulfiram in HIV-1-infected Adults on Antiretroviral Therapy

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BACKGROUND: Transcriptionally silent HIV-1 DNA persists in resting memory CD4⁺ T cells despite antiretroviral therapy. In a primary cell model, the anti-alcoholism drug disulfiram has been shown to induce HIV-1 transcription in latently infected resting memory CD4⁺ T cells at concentrations achieved *in vivo*. We hypothesized that disulfiram would be safe as a latency reversing agent *in vivo* and would result in a transient increase in plasma HIV-1 RNA levels due to virus release from latently infected T cells and a decline in the size of the latent reservoir.

METHODS: We conducted a single-arm pilot study to evaluate whether 500 mg of disulfiram administered daily for 14 days to HIV-1-infected individuals on stable suppressive antiretroviral therapy would result in reversal of HIV-1 latency demonstrated by change in residual viremia or depletion of the latent reservoir. Plasma was obtained weekly before and after the intervention period and three times weekly during the intervention to measure low level viremia using the single copy assay and disulfiram concentrations via mass spectrometry. A quantitative viral outgrowth assay was used to measure the frequency of latently infected T cells two weeks before and ten weeks after the intervention.

RESULTS: Disulfiram was safe and well tolerated. Disulfiram concentrations varied significantly from patient to patient. The latent reservoir did not change significantly (+1.16-fold change, 95% CI 0.70-1.92-fold, p=0.56). During disulfiram administration, residual viremia did not change significantly compared to baseline (+1.53-fold, 95% CI 0.88-2.69-fold, p=0.13). Over the two months following disulfiram administration, residual viremia was estimated to increase by 1.88-fold compared to baseline (95% CI 1.03-3.43-fold, p=0.04). This post-drug effect was more pronounced among the six participants with detectable disulfiram concentrations during drug administration (HIV-1 RNA increase +2.96-fold, 95% CI 1.29-6.81-fold, p=0.01).

CONCLUSIONS: Disulfiram was well tolerated by all participants. The size of the latent reservoir did not decrease after the intervention compared to baseline levels. The apparent exposure-response effect observed in this study highlight significant inter-subject variability in disulfiram pharmacokinetics and suggest that higher doses of disulfiram might be more effective. Disulfiram affects relevant signaling pathways and can be safely administered, and therefore may be useful in combination with other latency reversing agents.

ABSTRACT 135

HIV-1 Reactivation Primes the Latent Reservoir for Bcl-2mediated Eradication

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BACKGROUND: Histone deacetylase inhibitors (HDACi) selectively induce cancer cell death through mitochondrial apoptosis, a pathway that is regulated by a complex network of Bcl-2 family proteins. The extent to which transcriptional and immune activation stimuli alter apoptosis priming in nonneoplastic cells is unknown.

METHODS: We used BH3 profiling, a flow cytometrybased mitochondrial phenotyping assay, to explore the mitochondrial priming effects of activation stimuli on peripheral blood mononuclear cell (PBMC) subpopulations. PBMC isolated from HIV-infected, treated, virologically suppressed participants - defined as HIV-1 plasma RNA levels <50 copies/mL for \geq 1 year - were studied. Cells were incubated in the presence or absence of anti(a)-CD3/anti(a)-CD28 beads, vorinostat (SAHA), panobinostat (PNB), or romidepsin (RMD). Dose-ranging studies were performed and, after treatment with a standardized panel of death peptides, BH3 profiles were generated. A minimum of 3 experiments were performed for each condition.

RESULTS: Marked changes in the magnitude and phenotype of CD4+ T cell mitochondrial priming were observed in the presence of a-CD3/a-CD28 beads and the HDACi PNB and RMD, compared to untreated HIV-infected PBMC. BH3 profiles varied as a function of drug type, dose, and duration of exposure. Increased priming in response to the peptide BAD and decreased permeabilization to the peptide NOXAA was observed with both transcriptional and immune activating stimuli, signifying an increased dependence on the anti-apoptotic BCL-2 protein. The rank order of increased mitochondrial priming was RMD > PNB > a-CD3/a-CD28 beads >> SAHA. Similar magnitudes of priming occurred in central and transitional memory CD4+ T cell subsets. After exposure to RMD, follow-on Bcl-2 inhibition with the small molecule ABT-263 resulted in a dose-dependent increase in the proportion of annexin V-expressing CD4+ T cells, ranging from $3.5\% \pm 0.3\%$ (mean \pm SD) at RMD 20nM/ABT-263 1nM to $94.2\% \pm 2.7\%$ at RMD 350nM/ABT-263 1mM.

CONCLUSIONS: Histone deacetylase inhibition increased CD4⁺ T cell mitochondrial priming and cellular reliance on the anti-apoptotic protein Bcl-2. Follow-on Bcl-2 inhibition resulted in the near complete induction of T cell apoptosis. This two-hit combination therapeutics approach offers a strategy to directly purge the latent HIV reservoir that does not rely on immune memory.

ABSTRACT 136

Jak Inhibitors Tofacitinib and Ruxolitinib Employ a Novel Mechanism to Inhibit HIV-1 Replication and Multiple Pro-HIV/ Inflammatory Events in Human Cells

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BACKGROUND: With the success of HAART, the challenge now is to identify strategies to purge the latent reservoir to permit the discontinuation of antiretroviral therapy. The JAK-STAT pathway is activated in macrophages and lymphocytes upon HIV-1 infection, representing an attractive cellular target. Since tofacitinib and ruxolitinib are FDA-approved Jak1/2 inhibitors for treatment of rheumatoid arthritis and myelofibrosis, we evaluated them for their ability to block viral replication, pro-inflammatory cytokine-mediated activation of JAK/STAT signaling pathway(s), and inflammatory/ activation markers in T-cell-receptor-stimulated HIV-infected primary human lymphocytes and/or macrophages.

METHODS: Antiviral: Cells were treated with various concentrations of tofacitinib or ruxolitinib for 4hr prior to infection with HIV-1_{bal} (macrophages) or HIV-1_{NI4-3}. GEP (lymphocytes). Cells were maintained for 3 and 6 days before viral quantification (p24-ELISA or GFP, respectively). Inhibition: Macrophages were activated in m-CSF-containing media and differentiated for 14 days prior to stain with anti-CD163, CCR5, or HLA-DR in the presence of various concentrations of inhibitors. CD4 T-cells were isolated from viremic subjects and stimulated with anti-CD3/28 and increasing concentrations of inhibitors. After 6 days the following markers were measured by flow-cytometry (FCM): AnnexinV, CXCR4, CCR5, CFSE, CD25, CD38, HLA-DR. STAT-phosphorylation was measured by FCM in cells from healthy subjects stimulated for 15 min with IL-2, IL-6, IL-7, IL-10, IL-15 or IFN-[] and increasing concentrations of Jak inhibitors.

RESULTS: Tofacitinib and ruxolitinib were non-cytotoxic (> 50 μ M). Both drugs demonstrated 1) antiviral potency ranging from 0.02-0.08 μ M and 0.02-0.3 μ M in lymphocytes and macrophages, respectively, 2) significant reduction (p<0.05) in HIV-GFP-positive lymphocytes (EC50 0.1 μ M), 3) inhibition of anti-CD3/CD28 activation/proliferation in lymphocytes (CFSE-lo, CD38, CD25, HLA-DR), 4) 75% reduction in expression of CD163, HLA-DR/CCR5 expression in macrophages, and inhibition of IL-2, IL-7, IL-15, IL-10 or IFN- α induced STAT1, STAT3 or STAT5 phosphorylation in T-cells and/or monocytes (\geq 0.1 μ M).

CONCLUSIONS: Tofacitinib and ruxolitinib inhibited multiple pro-HIV/pro-inflammatory events in HIV-1 infected lymphocytes and macrophages, which are linked to viral persistence, trafficking of infected cells across the blood-brain-barrier/neurocognitive impairments, disease progression, and AIDS. Selectively blocking JAK/STAT signaling pathway(s) could reverse or prevent HIV-induced activation of macrophages and lymphocytes, thereby reducing HIV-orchestrated systemic immune activation and chronic inflammation, leading to a functional cure.

Effect of Combined Anti-latency Drugs on Cell Line J-Lat 82

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BACKGROUND: Pharmacological induction of latent viral reservoirs in conjunction with antiretroviral therapy is a potential approach to HIV persistence. We tested clinical or pre-clinical candidate compounds with proved anti-latency action and other drugs with mechanisms suitable to reactivate latent HIV on a latently infected cell line.

METHODS: We assessed the *in vitro* effect of drugs with several mechanisms of action, alone or combined, on GFP expression in the latent cell line J-Lat 82. We cultured 5X10⁵ cells on 24-well plates, in 1 ml of complete medium. GFP expression was measured by flow cytometry at 18 h, 24 h and day 3 after drug treatment.

RESULTS: HIV basal expression of J-Lat 82 was lower than other latent cell lines (0.35%). Positive controls (TNF, PMA, PMA-Ionomycin) potently induced viral reactivation. As individual treatment, Vorinostat, Prostratin and Bryostatin at different concentrations activated GFP expression when compared to mock-treated cells (2, 43 and 5.3 fold, respectively), while Inhibitors of DNA methylation Decitabine, Hydralazine, Procainamide and Disulfiram had no detectable effect. Additionally, we tested the effect of a drug with unclear mechanism of action on HIV reservoir, Auranofin, which had no effect on cells at 1 μ M but caused cell death at higher concentrations. As combined treatments, Vorinostat + Bryostatin showed an additive effect, while other combinations were associated wth GFP expression lower than expected.

CONCLUSION: This study describes the effect of several antilatency drugs, alone and combined, on the cell line J-Lat 82. The combination of drugs with several mechanisms of action on the latent proviral DNA is not necessarily associated with a synergistic effect.



Ma santé dès aujourd'hui, pour demain.



Kaletra 100 mg/25 mg, comprimé pelliculé. Kaletra 200 mg/50 mg, comprimé pelliculé. Kaletra (80 mg + 20 mg)/ml, solution buvable. COMPOSITION Kaletra 100 mg/25 mg : lopinavir : 100 mg + ritonavir : 25 mg pour 1 comprimé. Kaletra 200 mg/50 mg : lopinavir : 200 mg + ritonavir : 50 mg pour 1 comprimé. Kaletra solution buvable : 1 ml de solution contient 356,3 mg d'alcool (42,4 % v/v), 168,6 mg de sirop de maïs à haute concentration en fructose, 152,7 mg de propylène glycol (15,3 % m/v), 10,2 mg d'huile de ricin polyoxyl 40 hydrogénée et 4,1 mg d'acésulfame de potassium. Le ritonavir agit en potentialisant la pharmacocinétique du lopinavir. INDICATIONS THERAPEUTIQUES Kaletra est indiqué en association avec d'autres médicaments antirétroviraux pour le traitement des adultes, des adolescents et des enfants âgés de plus de deux ans infectés par le virus de l'immunodéficience humaine (VIH-1). Chez les patients infectés par le VIH-1 et déjà traités par des inhibiteurs de protéase, le recours au Kaletra devrait être basé sur les résultats des tests individuels de résistance virale et sur l'historique du traitement des patients (se reporter au RCP, rubriques 4.4 et 5.1). POSOLOGIE ET MODE D'ADMINISTRATION Prescription par des médecins expérimentés dans la prise en charge de l'infection par le VIH. Kaletra comprimé doit être avalé en entier sans être ni mâché, ni coupé, ni broyé. Posologie : en fonction de l'âge, du poids et/ou de la surface corporelle (se référer aux conditions prévues par le RCP). La forme solution buvable est recommandée chez l'enfant pour obtenir la posologie la plus précise, en fonction de la surface corporelle. *Enfants de moins de 2 ans :* la sécurité d'emploi et l'efficacité n'ont pas encore été établies. Aucune recommandation posologique proposée. CONTRE-INDICATIONS Hypersensibilité aux substances actives ou à l'un des excipients. Insuffisance hépatique sévère.

J'aimerais...

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ENSEIGNE

FAIRE DU SPORT

