# Highlights from the Conference on Retroviruses and Opportunistic Infections 2016
## 22–25 February 2016, Boston, Massachusetts, USA

## Analytical treatment-interruption trials

Sarah Fidler[1] and Jintanat Ananworanich[2]

[1] *Imperial College London, UK*
[2] *US Military HIV Research Program, Walter Reed Army Institute of Research; Henry M Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA*

The ultimate test of HIV remission is maintenance of undetectable plasma viraemia off antiretroviral therapy. Several studies, both past and recent have explored how common this phenomenon is and what factors predict successful viral control where present. There is no unifying definition of post-treatment control (PTC), in terms of length of time plasma viral load (VL) remains below a certain threshold and comparison between studies demonstrates a variable definition of the phenotype. Table 1 summarises studies presented at the meeting where individuals were enrolled into a carefully monitored analytical treatment interruption (ATI) strategy. Overall, despite many years of successful viral suppression and CD4 recovery, PTC was uncommon. Where virus rebounded, this occurred generally within the first 2–8 weeks after ATI

The ULTRASTOP study selected 10 participants who initiated antiretroviral therapy (ART) in early chronic HIV infection and exhibited tight viral control with low HIV RNA and DNA, and high CD4 and CD4/CD8 ratio. Half of the cohort had protective HLA alleles associated with elite controllers and PTC status. Despite these favourable qualities, nine of 10 participants had a rapid viral load rebound post-ATI. One participant has controlled viraemia below 400 copies/mL for over 1 year. He possesses HLA-B2703 that could have contributed to the prolonged viraemic control, although others with such HLA profile in this cohort did not [1,2].

The pooled ACTG analyses examined the relationship between time to VL rebound and VL set point post-ATI. They observed that longer time to VL rebound and earlier ART initiation was associated with lower VL set point. VL set point in these studies was defined as the mean plasma viral load measured in HIV RNA copies/mL taken 12–16 weeks after TI. VL set points were similar with non-nucleoside analogue- and protease inhibitor-based regimens. In chronically treated individuals, pre-ART VL was positively associated with levels of VL set point. Interestingly, set point VL was two-fold lower than pre-ART VL, which cautions against using lower VL levels at set point compared to pre-ART as an indicator for efficacy of interventions in studies without a control arm [3,4].

The SPARTAC investigators presented data from the African SPARTAC cohort that enrolled 82 females from sub-Saharan Africa with subtype C primary HIV infection. Participants were randomly allocated to no ART ($n$=38) vs 48 weeks of ART ($n$=44). Of those treated for 48 weeks, 22 with VL <400 copies/mL underwent ATI and five achieved PTC status at week 48 (PTC rate of 22%) [5]. This is higher than that observed in the UK SPARTAC cohort (PTC rate of 5%) [6]. However, the predictors for time to VL rebound in the UK cohort were not replicated here (total HIV DNA and markers of immune exhaustion) [7,8]. These findings illustrate the potential contribution of host (gender, ethnicity) and viral subtype on HIV remission, and highlight the limitation of extrapolating findings across populations.

Interventions in addition to ART amongst chronically infected individuals with years of sustained viral suppression were also presented. Two studies presented findings following infusion of the broadly neutralising antibody (bNAb) VCR01 [9,10]. VRC01 targets the CD4 binding site of HIV Env and neutralises ~90% of HIV isolates with high potency (mean $IC_{50}$ 0.33 µg/mL, mean $IC_{80}$ 1 µg/mL). In both studies, infusion of the antibody was safe and well tolerated, and achieved plasma levels of antibody at least 50-fold above the $IC_{80}$. In the ACTG5340 study, 13 chronically HIV-infected males with long-term viral suppression received VRC01 1 week prior to ATI and every 3 weeks post-ATI. All experienced viral rebound >200 copies/mL, and in the majority, it occurred rapidly within the first 5 weeks. There was a trend towards delayed initial rebound with 38% of participants in this study maintaining viral suppression at week 4 compared to 13% in the historical ACTG cohort without any intervention [11], but this did not persist after week 4. In the NIAID study, 10 participants who had been VL suppressed for about a decade interrupted ART after receiving VRC01 3 days prior and every 2–4 weeks subsequently. All except one rebounded at a median time of 39 days. Pre-existing and rapid emergence of VRC01 was likely to contribute to the failure of VRC01 to suppress viraemia. These findings suggest that in chronic HIV infection, combination bNAbs will probably be needed to control viraemia post-ATI.

In the combination intervention pilot study amongst chronically infected virally suppressed individuals in the Reduc study, participants received Vacc-4x vaccination [12] with RhGM-CSF adjuvant, followed by a latency-reversing agent (LRA), romidepsin [13]. The regimen was safe and well tolerated. Eight of 17 participants showed detectable viraemia with romidepsin infusions. Data on 6/17 individuals enrolled showed a modest reduction in total HIV DNA of 40% and in viral outgrowth assay of 36% after completion of the intervention. However, subsequent ATI led to all participants experiencing rapid viral rebound to >50 copies/mL HIV RNA by day 14. The vaccine did not appear to generate HIV-specific CD8 immune responses [14].

An interesting study in rhesus macaques showed that toll-like receptor (TLR)-7 agonist given in addition to ART could lead to simian immunodeficiency virus (SIV) remission. In 11 rhesus monkeys infected with SIVmac251 and initiated on ART at day 65 post-infection, different doses of TLR7 agonist ($n$=9) vs placebo ($n$=2) were given starting at day 467 post-infection. SIV DNA levels were reduced in memory CD4+ T cells in TLR7-agonist treated monkeys on ART. In seven of nine TLR7-agonist treated animals, reductions of inducible RNA were observed. Of interest, two animals had undetectable inducible virus from blood and lymph node cells before stopping ART, and they did not experience viral rebound after more than 3–4 months off ART [15].

These studies constitute promising work that highlights the difficulty in achieving the goal of HIV remission and cure. Earlier initiation of ART can prolong time to VL rebound, but by itself, will not be enough to ensure that the majority of people can achieve remission. Combination therapies will be needed and may include potent latency-reversing agents, two or three

**Table 1.** Analytical treatment interruption studies with and without immunological interventions

| Study | Design | Primary outcomes | Participants | Findings |
|---|---|---|---|---|
| **Without immune interventions** | | | | |
| ULTRASTOP [2] | ATI in early treated chronically infected persons with low HIV DNA and high CD4 | Frequency of PTC (VL <400 copies/mL post-ATI) | 10 participants (7 male) with median CD4 1118 cells/mm$^3$, CD4/CD8 ratio 0.9 and HIV DNA <66 copies/$10^6$ PBMCs with median time on ART of 5 years. Five were either HLA-B27 or HLA-B57 positive | 1/10 had VL <400 copies/mL at week 48 post-ATI (PTC rate 10%, 95% CI 0.3–44.5). This participant had CCR5 wild-type virus and was positive for HLA-B2703, HLA-B4001. 9/10 had VL rebound at week 2 ($n$=2), week 4 ($n$=6) and week 12 ($n$=1). Median (range) time to VL rebound was 4 (2–12) weeks |
| ACTG [4] | Pooled analysis of six ACTG ATI studies | Association between time to VL rebound ≥200 copies/mL and VL set point (mean VL between weeks 12–16) | 235 participants (91% male) on suppressive ART initiated in recent (34%) and chronic HIV (66%) infection. Median time on ART was 5.1 years | 14 participants with VL rebound >12 weeks post-ATI had lower VL set point than the 180 who rebounded at ≤12 weeks post-ATI (median 1.9 $vs$ 4.1 log$_{10}$ copies/mL, $P$<0.001). Participants treated in recent HIV infection had lower VL set point than those treated later (median 3.5 $vs$ 4.2 log$_{10}$ copies/ml, $P$<0.001). In chronically treated individuals, pre-ART VL was associated with VL set point level but not time to VL rebound. VL set point was two-fold lower than pre-ART VL |
| SPARTAC [5] | Randomised trial of no ART $vs$ 48 weeks during primary HIV infection followed by ATI | Frequency of PTC (VL <400 copies/mL post-ATI) | 22 female participants from sub-Saharan Africa with subtype C HIV | 5 of 22 African participants had VL <400 copies/mL post-ATI (PTC rate 22%) over a median of 188 weeks (range 147–203). No predictive biomarkers of time to VL rebound were observed |
| **With immune interventions** | | | | |
| ACTG5340 [9] | Single-arm study of VRC01 bNAb during ATI in chronically treated individuals. VRC01 40 mg/kg/dose given at −1 week, and q 3 weeks post-ATI | Safety, tolerability, plasma VRC01 level. Time to VL rebound >200 copies/mL | 13 male participants on suppressive ART initiated in chronic HIV. Median of 4.7 years on ART | VRC01 was safe and well tolerated. Plasma VRC01 levels were >50 μg/mL post-ATI (mean IC$_{80}$ of VRC01 is 1 μg/ml). All participants had VL rebound (11 by week 5, one by week 8 and one by week 11). 38% in this study $vs$ 13% in a historic cohort remained with VL suppression at week 4, $P$=0.04. Rebound virus displayed clonal selection and pre-existing resistance |
| NIAID [10] | Single arm study of VRC01 bNAb administered at day −3 and weeks 2, 4 and q 4 weeks post-ATI | Safety, tolerability, plasma VRC01 level. Time to VL rebound >40 copies/mL | 10 participants on suppressive ART initiated in chronic HIV. Mean of 10.6 years on ART. | VRC01 was safe and well tolerated. All had high plasma concentrations of VRC01. 9/10 experienced viral rebound >40 copies/mL between 11–86 days (median 39 days). Pre-existing and rapid emergence of VRC01-resistant viruses likely contributed to viraemia |
| Reduc [14] | Single arm study of Vacc-4x (four modified peptides to p24) 1.2 mg ID with RhGM-CSF adjuvant 0.06 mg ID given at days 0, 7, 14, 21, 77 and 84 plus romidepsin 5 mg/m$^2$ IV at days 105, 112 and 119, followed by ATI on day 182 | Safety, tolerability. Time to VL >50 copies/mL | 20 participants (17 males) on suppressive ART initiated in chronic HIV infection. Median time on ART was 6.3 years. Median total HIV DNA at baseline was 1080 copies/$10^6$ CD4 | The combination was safe and well tolerated. All participants had VL rebound >50 copies/mL at a median time of 14 days post-ATI. 8/17 (47%) had detectable plasma viraemia during romidepsin infusions. Prior to ATI, data on 6/17 participants showed a mean reduction of total HIV DNA of 39.7% (95% CI: −11.5– −58.9) and for viral outgrowth assay (IUPM), it reduced by 38% (95% CI: −67– −8). HIV-specific CD8 immune responses were similar before and after the intervention |

ATI: analytical treatment interruption; PTC: post-treatment controller; PBMC: peripheral blood mononuclear cells; VL: viral load; bNAb: broadly neutralising antibody

bNAbs with different targets and/or therapeutic vaccines that generate strong HIV-specific immune responses.

## Acknowledgements

## References

1. Calin R, Hamimi C, Lambert-Niclot S *et al.* Treatment interruption in chronically HIV-infected patients with an ultralow HIV reservoir. *AIDS* 2016; **30**: 761-769.

2. Hamimi C, Lambert S, Calin R *et al.* Role of transitional-memory T cells in productive HIV reservoir in ULTRASTOP patients. *Conference on Retroviruses and Opportunistic Infections.* February 2016. Boston, MA, USA. Abstract 332.

3. Treasure GC, Aga E, Bosch RJ *et al.* Relationship among viral load outcomes in HIV treatment interruption trials. *J Acquir Immune Defic Syndr* 2016.

4. Treasure GC, Aga E, Bosch R *et al.* Relationship among viral load outcomes in HIV treatment interruption trials. *Conference on Retroviruses and Opportunistic Infections.* February 2016. Boston, MA, USA. Abstract 346.

5. Gossez M, Ramjee G, Hurst J *et al.* Virological remission after ART interruption in African HIV-1 seroconvertors. *Conference on Retroviruses and Opportunistic Infections.* February 2016. Boston, MA, USA. Abstract 87.

6. Fidler S, Porter K, Ewings F *et al.* Short-course antiretroviral therapy in primary HIV infection. *N Engl J Med* 2013; **368**: 207-217.

7. Hurst J, Hoffmann M, Pace M *et al.* Immunological biomarkers predict HIV-1 viral rebound after treatment interruption. *Nat Commun* 2015; **6**: 8495.

8. Williams JP, Hurst J, Stohr W *et al.* HIV-1 DNA predicts disease progression and post-treatment virological control. *Elife* 2014; **3**: e03821.

9. Bar KJ, Harrison LJ, Overton HE *et al*. ACTG 5340: the effect of VRC01 on viral kinetics after analytic treatment interruption. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA. Abstract 32LB.

10. Chun TW, Sneller M, Seamon C *et al*. Effect of infusion of broadly neutralizing antibody VRC01 on HIV plasma rebound. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA. Abstract 311LB.

11. Li JZ, Etemad B, Ahmed H *et al*. The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption. *AIDS* 2016; **30**: 343-353.

12. Pollard RB, Rockstroh JK, Pantaleo G *et al*. Safety and efficacy of the peptide-based therapeutic vaccine for HIV-1, Vacc-4x: a phase 2 randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis* 2014; **14**: 291-300.

13. Sogaard OS, Graversen ME, Leth S *et al*. The depsipeptide romidepsin reverses HIV-1 latency *in vivo*. *PLoS Pathog* 2015; **11**: e1005142.

14. Leth S, Højen J, Schleimann M *et al*. Effect of sequential Vacc-4x/GM-CSF immunization and romidepsin on the HIV reservoir. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA. Abstract 26LB.

15. Whitney JB, Lim SY, Osuna CE *et al*. Repeated TLR7 agonist treatment of SIV+ monkeys on ART can lead to viral remission. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA. Abstract 95LB.

---

# Reservoirs, relapse and remission

Linos Vandekerckhove and Maja Kiselinova

*Department of Internal Medicine, Ghent University Hospital, Belgium*

## Investigating the mechanisms that control HIV transcription and latency *in vivo*

The mechanisms that maintain HIV latency *in vivo*, and the role of transcriptional interference in limiting HIV transcription are yet unclear. Yukl *et al.*'s hypothesis was to investigate whether levels of different HIV RNA transcripts could be used to infer the sites of transcriptional blockade [1]. To answer this hypothesis they used CD4+T cells from nine ART-suppressed individuals, which were subsequently activated for 2 days with anti-CD3/CD28 beads or not activated. The purified RNA from the samples was than assayed with ddPCR for specific and different HIV transcripts indicative of: (1) transcriptional interference (U3-U5; 'read-through'); (2) initiation (TAR); (3) elongation (R-U5-tRNA; 'long'); (4) completion (3′LTR-polyA; 'polyA'); and (5) multiple splicing (tat-rev). The group found a relative abundance of HIV transcripts in unstimulated CD4+T cells, indicating blocks to proximal elongation, completion and splicing. Hence, in unstimulated CD4+T cells the transcriptional interference was found to play a modest role in limiting HIV transcription, while blocks in elongation, completion and splicing dominated. In contrast, the CD4 T cell activation was found to increase all transcripts. More specifically, activation was found to selectively increased distal and spliced transcripts but had less effect on read-through or total transcripts, suggesting that the main reversible blocks to HIV expression are not interference or lack of initiation, but rather inhibition of elongation/ polyadenylation and splicing.

## Human galectin-9 is a potent mediator of HIV transcription and reactivation

HIV transcription, more specifically host determinants that govern HIV transcription and latency are one of the several research focuses in HIV cure studies. In this sense and based on previous findings, Abdel-Mohsen *et al.* [2] presented new data on glycan-binding protein galectin-9 (Gal-9) and its mediation in HIV transcription and reactivation. The same group previously showed that human Gal-9 regulates p21 (the host factor) and in turn p21 regulates HIV transcription during antiretroviral therapy (ART). Therefore, for this research they hypothesised that Gal-9 modulates HIV transcription. To test this hypothesis they used plasma from 72 HIV-infected ART-suppressed individuals. In addition, by using the J-Lat 5A8 HIV latency model and primary CD4+ T cells isolates from 13 HIV-infected ART-suppressed individuals, the group

evaluated the ability of a recombinant, stable form of Gal-9 (rGal-9) to reactivate latent HIV. In addition, the group used the enzymatic and chemical deglycosylation to explore the requirement of glycans in viral reactivation by rGal-9. In summary, their findings indicated that the cell surface N-linked oligosaccharides and O-linked hexasaccharides were essential for rGal-9-induced HIV reactivation, mediated by key transcription initiation, promoter proximal-pausing, and chromatin remodelling factors. More importantly, rGal-9 was found to reactivate latent HIV more potently than anti-CD3/CD28 stimuli and to induce expression of the host antiviral deaminase APOBEC3G *in vitro* and *ex vivo*. Hence, rGal-9-induced virus is suggested to be replication incompetent as a result of APOBEC3G induction in the producer cell, which than will ensure that the reservoir will not be replenished when latency is reversed therapeutically, even in the setting of suboptimal ART suppression.

## 1PD-1+ and Tfh cells represent the major source of HIV-1 replication-competent virus

In the past few years, research has shown that T follicular helper (Tfh) cells are the major CD4 T cell compartments for HIV infection and replication, specifically in viraemic HIV-1-infected individuals. Banga *et al.* [3] asked the question of what is the role of Tfh in long-term treated aviraemic HIV-1 patients. Hence, they investigated the distribution of CD4 T cells containing replication-competent virus within CXCR5-PD-1-, CXCR5+PD-1- and PD-1+ memory CD4 T cell populations isolated from blood and lymph nodes (LN) of 11 long-term treated (1.5–14 years) aviraemic subjects. The results revealed that the levels of HIV-1 RNA produced in LN PD-1+ CD4 T cells were higher (810–1225 fold higher) as compared to blood and were found to be inversely correlated with the duration of treatment. Interestingly, they found that HIV-1 produced by LN PD-1+ CD4 T cells was also infectious when tested *in vitro* with CD4 T cells of HIV-negative subjects. Hence, these findings showed that LN PD-1+ CD4 T cells were enriched with replication-competent virus and that these cells represent the major source of replication-competent, infectious virus in long-term treated aviraemic HIV-1-infected individuals.

## Rapid accumulation of defective proviruses complicates HIV-1 reservoir measurements

It is well known that defective proviruses predominate in resting CD4+ T cells from patients treated with combined antiretroviral therapy. Brunner *et al.* [4] investigated if the fraction of defective proviruses is constant or increases over the course of the infection. This could be supported by the idea that cells with genetically intact proviruses are eliminated during suppressive therapy. To answer this question an *in vitro* infection of CD4+ T cells was performed to determine the fraction of defective proviruses after a single round of infection. In addition proviruses from patients treated in either acute or chronic infection and untreated viraemic patients were characterised. Proviruses were analysed by an unbiased, limiting dilution, full genome PCR and direct sequencing of PCR products. The number of intact proviruses was quantified as a percentage for each patient and compared to total HIV-1 DNA ddPCR values and quantitative viral outgrowth assay (QVOA) IUPMs. Defective proviruses made up 40% of proviruses in the one round *in vitro* replication set up. Moreover, less than 5% of proviruses were intact in both patient groups; the remaining proviruses contained major defects. In a comparative study, the QVOA underestimates the latent reservoir by a median of 64-fold in chronically treated patients and 20-fold in acutely treated patients while total DNA PCR measurements vastly overestimated the reservoir in both patient groups. An additional finding was that during acute infection, the fraction of defective proviruses is likely

to increase very rapidly, quickly making up over 95% of proviruses in HIV-1 patients.

## Clones of SIV-infected cells are present in spleen and lymph nodes in rhesus macaques

Because there are limits on the samples that can be obtained from patients, Ferris *et al.* [5] developed a model using SIV-infected rhesus macaques. An integration site library from rhesus macaque PBMCs infected in culture with SIV was generated. In addition six additional libraries were generated from two rhesus macaques that were infected with SIV for 4 weeks and then treated for approximately 1 year with a cART regiment. One important finding was that the distribution of the SIV integration sites in the large integration site library (~50,000 independent sites) prepared from the rhesus macaque PBMC infected *in vitro* was quite similar to the distribution of HIV integration sites in human PBMCs. The group identified the presence of expanded clones in two SIV macaques that were treated after 4 weeks of infection indicating that cells that are infected early can give rise to expanded clones. Cells from two clones were present in both lymph node and spleen, showing that the distribution was not tissue restricted. In this first experiment, there was no evidence for the selection of cells that have integration sites in either BACH2 or MKL2.

## ART reduces cellular HIV RNA but not the fraction of proviruses transcribing RNA

Hong *et al.* [6] performed an interesting study investigating both the fraction of infected cells that express HIV RNA and the levels of HIV RNA in single cells from untreated, viraemic individuals and from those on suppressive ART. PBMCs from five viraemic (median VL=5727 copies/mL) and five ART-suppressed (VL<20 copies/mL) individuals were analysed for expression of unspliced viral RNA in single cells using two methods: cell-associated RNA (CAR) and DNA single-genome sequencing (CARD-SGS), and fractional proviral expression (fPVE). Importantly, the proportion of the proviruses that expressed unspliced CAR was not different between viraemic and ART-suppressed individuals (median 7% *vs* 6%, respectively). By contrast, the fraction of cells that were 'high CAR producers' (>20 CAR copies/cell) was greater in viraemic than suppressed individuals. The differences in HIV RNA expression levels in single cells between ART-suppressed and viraemic individuals suggest that cells producing high levels of HIV RNA are associated with active virus replication and are eliminated by viral CPE or CTL responses, whereas the more frequent cells expressing low levels of HIV RNA can persist and expand despite ART.

## Restricted HIV-1 diversity and clonal expansion following cytoreductive chemotherapy

Cytoreductive chemotherapy for malignancies does not lead to consistent changes in HIV-1 DNA or RNA. However, observed reductions in CD4 T cells during chemotherapy suggest that total body reservoirs decrease. Henrich *et al.* [7] evaluated whether constriction and subsequent oligoclonal expansion of HIV diversity may be a better measure of the reservoir response to novel HIV eradication strategies. Longitudinal, single-genome analysis of HIV envelope sequences was performed in a cohort of 10 infected individuals on suppressive ART receiving chemotherapy. Although CD4 T cell counts transiently decreased by up to 75% during chemotherapy, CD4 T cell HIV DNA did not change and RNA increased following completion of therapy. Clonal expansion of HIV-1 envelope sequences following chemotherapy was observed in three of six patients for whom data was obtained; sequence clustering was only seen following completion of chemotherapy. Importantly, CD4 T cells that responded to EBV/CMV lysates had

higher HIV-1 DNA levels compared to those that responded to αCD3/αCD28 stimulation or did not express IL2/IFN-γ. Hence, these data also suggest that response to non-HIV antigens can lead to oligoconal expansion of the DNA reservoir.

## References

1. Yukl S, Kaiser P, Kim P *et al.* Investigating the mechanisms that control HIV transcription and latency *in vivo*. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA.

2. Abdel-Mohsen M, Chavez L, GM C *et al.* Human galectin-9 Is a potent mediator of HIV transcription and reactivation. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA.

3. Banga R, Procopio F, Cavassini M *et al.* PD-1+ and Tfh cells represent the major source of HIV-1 replication-competent virus. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA.

4. Brunner KM, Pollack R, Murray A *et al.* Rapid accumulation of defective proviruses complicates HIV-1 reservoir measurements. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA.

5. Ferris A, Del Prete GQ, Keele B *et al.* Clones of SIV-infected cells are present in spleen and lymph nodes in rhesus macaques. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA.

6. Hong F, Spindler J, Musick A *et al.* ART Reduces Cellular HIV RNA but Not the Fraction of Proviruses Transcribing RNA. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA.

7. Henrich TJ, Scully EP, Hobbs KS *et al.* Restricted HIV-1 diversity and clonal expansion following cytoreductive chemotherapy. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA.

---

# The microbiome affects everything

Alexandra Schuetz

*Department of Retrovirology, US Army Medical Component, Armed Forces Institute of Medical Research, Henry M Jackson Foundation, Bangkok, Thailand*

Microbes, in the setting of HIV infection, are likely to be playing a large role in contributing to HIV-1 pathogenesis, morbidities and mortality. Two major disruptions to microbial systems in HIV-1 infection include microbial translocation (MTL) and microbiome dysbiosis [1]. MTL occurs when bacteria (or bacterial products) translocate from the lumen of the intestine across the epithelial barrier into systemic circulation, where they contribute to inflammation and pathogenesis. In rhesus macaques, MTL begins during the late acute phase of SIV infection and increases during chronic infection and is associated with structural damage of the gastrointestinal (GI) tract. Moreover, immune activation is temporally and causally related to MTL and the relative inability of intestinal macrophages to bind/phagocytose translocated microbial products [2]. In addition, microbial populations in the GI tract are also altered after HIV infection, resulting in microbiome dysbiosis, which further exacerbates MTL, epithelial barrier disruption, inflammation and mucosal immune functioning. A dysbiotic mucosal-adherent community enriched in Proteobacteria, among them many pathogens, and depleted of Bacteroidetes and Firmicutes is associated with disruption of mucosal barriers, immune activation and chronic inflammation in HIV-1 infected patients [3,4].

During chronic HIV-1 infection, microbial products migrate into the blood from the GI tract concomitant with systemic immune activation. Ericsen *et al.* [5] reported that within the first week of SIV infection (hyperacute infection) plasma levels of bacterial DNA increased transiently as much as 1300-fold and plasma genera detected in the stool up to 40% over baseline. In addition, he also observed an increase of circulating IFABP, a marker of enterocyte loss, suggesting that there is permeability within the GI lining. Plasma levels of soluble CD14 (sCD14) correlated with set-point levels of virus replication. In addition, this hyperacute MTL was accompanied by peripheral inflammation and an increase in peripheral CD4+CCR5+ T cells, suggesting that this might promote early virus replication.

During the themed discussion lead by Nichole Klatt, Luévano *et al.* detailed [6] the impact of HIV-associated changes in the gut microbiome on disease progression. Based on faecal material from HIV-uninfected subjects, his data demonstrated a high intra-subject bacterial community variability (richness) and a unique population primarily composed of Firmicutes. During HIV-1 infection significant changes in the gut microbiome were observed, showing a decrease in richness and change in composition with a decrease of Firmicutes and an increase of Bacteroidetes. With antiretroviral treatment those changes were less significant, but did not return to those observed in HIV-uninfected individuals. These alterations suggest that the enteric microbiome is significantly altered by HIV-1 infection and may directly contribute to disease progression.

From there the discussion moved on to the effect of the microbiome on HIV-1 induced immunity with Dillon *et al.* [7] showing how an altered gut microbiome enhances mucosal CD4 T cell infection and depletion *ex vivo*. Employing a model using lamina propria mononuclear cells (LPMC) isolated from human jejunum she showed that the majority of HIV-altered mucosal bacteria (HAMB) increased HIV-1 infection and depletion of LP CD4 T cells. However, Gram-negative HAMB enhanced CD4 T cell infection to a greater degree than Gram-positive HAMB. In this context, lipopolysaccharide, a Gram-negative bacteria cell-wall component, upregulated CCR5 expression on LP CD4 T cells whereas Gram-positive cell wall lipoteichoic acid did not. Thus enhanced infection appears to be primarily mediated indirectly through increased expression of CCR5 on LP CD4 T cells without concomitant large-scale T cell activation. This represents a novel mechanism potentially linking intestinal dysbiosis to HIV-1 mucosal pathogenesis and is in concordance with the data shown by Ericsen *et al.* [5].

The themed discussion closed with a presentation on how the gut microbiome can be modulated during HIV-1 infection to improve disease outcome. Somsouk *et al.* [8] presented data on a faecal microbial transplantation (FMT) study aimed at reversing HIV-1 induced dysbiosis. In this open-label FMT study of six HIV-positive, ART-suppressed participants the donor microbiome, enriched with Bacteroidetes and Faecalibacterium was delivered by colonoscopy. After FMT, the recipient microbiome significantly shifted towards the donor microbiome indicated by UniFrac distance, showing the similarity between microbiobial communities. The degree of microbial engraftment was modest with a single delivery of FMT, and the effect on inflammation remains to be determined.

Taken together, even though it is clear that HIV-1 infection damages the GI mucosa leading to MTL, which is accompanied by intestinal dysbiosis, it remains under examination if dysbiosis is the cause or rather the symptom. This makes it imperative to better understand the complex relationships between the gut microbiome and mucosal dysfunction to develop new therapeutic strategies in the management of chronic HIV and, potentially, other chronic inflammatory conditions.

## References

1. Zevin AS, McKinnon L, Burgener A, Klatt NR. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. *Curr Opin HIV AIDS* 2016; **11**: 182–190.
2. Estes JD, Harris LD, Klatt NR *et al.* Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. *PLoS Pathog* 2010; **6**: e1001052.
3. Vujkovic-Cvijin I, Dunham RM, Iwai S *et al.* Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med* 2013; **5**: 193ra191.
4. Dillon SM, Lee EJ, Kotter CV *et al.* An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. *Mucosal Immunol* 2014; **7**: 983–994.
5. Ericsen AJ, Lauck M, Mohns MS *et al.* Hyperacute microbial translocation during pathogenic SIV infection. *Conference on Retroviruses and Opportunistic Infections.* February 2016. Boston, MA, USA. Abstract 21.
6. Luévano J, Gootenberg DB, Paer JM *et al.* Impact of HIV-associated changes in the gut microbiome on disease progression. *Conference on Retroviruses and Opportunistic Infections.* February 2016. Boston, MA, USA. Abstract 261.
7. Dillon SM, Lee EL, Donovan AM *et al.* Altered gut microbes enhance mucosal CD4 T cell infection and depletion *ex vivo. Conference on Retroviruses and Opportunistic Infections.* February 2016. Boston, MA, USA. Abstract 262.
8. Somsouk M, Vujkovic-Cvijin I, Pao M *et al.* Fecal microbial transplantation: safety and engraftment during treated HIV infection. *Conference on Retroviruses and Opportunistic Infections.* February 2016. Boston, MA, USA. Abstract 264.

# Interventional studies for complications of HIV disease: from bench toward the clinic

Jaime H Vera

*Lawson Unit, Brighton and Sussex University NHS Trust and Brighton and Sussex Medical School, UK*

The introduction of combination antiretroviral therapy (cART) has transformed HIV disease into a manageable long-term chronic condition. Despite this, there is convincing evidence that compared to the general population people living with HIV (PLWH) have an increased rate of comorbidities such as cognitive impairment, cardiovascular, liver and bone disease. The reasons for this are multifactorial including immune activation, antiretroviral therapy, specific lifestyle and the HIV virus itself [1]. There is an urgent need for novel treatment strategies to tackle non-AIDS comorbidities of PLWH on effective cART as well as those commencing cART. A major theme of the scientific presentations at the 2016 CROI was the investigation of novel interventions for the management of complications in HIV disease.

HIV-associated cognitive disorders (HAND) are frequently reported in PLWH on effective cART, and have been strongly associated with persistent central nervous system inflammation [2]. Although adjunctive therapies are likely to play a role in the management of HAND, medication treatment strategies for HAND have been disappointing. Sacktor and colleagues [3] presented findings from a trial of paroxetine and fluconazole therapy for HAND. After screening over 2000 approved compounds for neuroprotection, they selected fluconazole and paroxetine because of their potent immunoprotective effects in *in vitro* and SIV models of mitochondrial stress and neuronal damage. Forty-five HIV-positive individuals with evidence of cognitive impairment were enrolled in a 24-week randomised double-blind placebo-controlled trial where participants were assigned to one of four groups: (1) paroxetine 20 mg daily; (2) fluconazole 100 mg twice a day; (3) paroxetine plus fluconazole; and (4) placebo. The study showed benefit in cognitive performance in HIV-positive individuals in the paroxetine arms (alone or in combination with fluconazole) compared to the non-paroxetine arms, after adjusting for depression. There was no benefit in cognitive performance in HIV-positive individuals on the fluconazole arms; however, fluconazole was associated with a reduction of CSF ceramide, a marker of oxidative stress. Interestingly, cognitive improvement in the paroxetine arms was not associated with reductions in CSF lipid markers of oxidative stress. The investigators concluded that paroxetine might be associated with cognitive improvement in patients with HAND. Although a larger study is needed to corroborate the findings, these results are encouraging as they show a beneficial effect of adjunctive therapy for HAND in a randomised controlled trial. In another translational medicine study, Ofotokun and colleagues [4] demonstrated in a randomised double-blind placebo-controlled trial that a single infusion of zolendronic acid at the time of ART initiation prevented ART-induced bone resorption and bone mineral density (BMD) loss in HIV-positive individuals initiating cART (atazanavir/ritonavir +

tenofovir/entricitabine) after 48 weeks. It is well established that HIV infection is an independent risk factor for bone mineral loss, and that initiation of cART can induce additional loss in bone mineral density (BMD)[5]. BMD loss in the context of HIV occurs early, and irrespective of the type of cART, suggesting a mechanistic role of immune reconstitution. Using a mouse model of HIV immune reconstitution the authors previously demonstrated that zolendronic acid significantly inhibited immune reconstitution, preventing bone resorption and mineral loss [6]. They have now moved a step forward and demonstrated the potential of using zolendronic acid prophylactically to prevent BMD loss associated with both HIV and initiation of cART.

In summary, findings from these translational clinical studies, if replicated in larger clinical trials, are a step forward in the search for management strategies for non-AIDS comorbidities affecting PLWH.

## References

1. Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. *Lancet* 2013; **382**: 1525–1533.

2. Heaton RK, Clifford DB, Franklin DR, Jr. *et al.* HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* 2010; **75**: 2087–2096.

3. Sacktor N, Skolasky RL, Haughey N *et al.* Paroxetine and fluconazole therapy for HAND: a double-blind, placebo-controlled trial. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA. Abstract 146.

4. Ofotokun I, Titanji K, Vunnava A *et al.* A single dose zoledronic acid prevents antiretroviral-induced bone loss. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA. Abstract 47.

5. Ofotokun I, Titanji K, Vunnava A *et al.* Antiretroviral therapy induces a rapid increase in bone resorption that is positively associated with the magnitude of immune reconstitution in HIV infection. *Aids* 2016; **30**: 405–414.

6. Ofotokun I, Titanji K, Vikulina T *et al.* Role of T-cell reconstitution in HIV-1 antiretroviral therapy-induced bone loss. *Nature communications* 2015; **6**: 8282.

## LATTE 2 study

Ellen Dwyer

*St George's University Hospitals NHS Foundation Trust, London, UK*

Over the past 20 years the contents of the HIV physician's treatment toolbox have improved dramatically. Patients now have access to an array of antiretroviral medications with low side-effect profiles that require only once-daily dosing, often in single-tablet combinations. Despite significant treatment advances, the impact of antiretroviral therapy is limited by incomplete adherence, a major cause of treatment failure, and the emergence of pharmacological resistance.

In other fields of medicine, including psychiatry and reproductive health, improvements in adherence have emerged through the use of long-acting injectables [1]. Similar agents may offer a more convenient method of antiretroviral administration, potentially improving adherence and patient satisfaction.

David Margolis' presentation of the 32-week results from the LATTE-2 (Long-Acting Antiretroviral Treatment Enabling) trial [2] described a major advance in the development of injectable antiretroviral agents. It analysed a combination injectable containing the integrase inhibitor cabotegravir (previously GSK 744) and the non-nucleoside reverse transcriptase inhibitor rilpivirine. The main objectives were to investigate the safety and efficacy of this regimen in treatment maintenance, and establish an appropriate dosing schedule for progression into Phase III studies. The efficacy of the oral cabotegravir/rilpivirine combination had been studied in LATTE-1 [3], and was shown to be non-inferior to efavirenz and two nucleoside reverse transcriptase inhibitors in a 72-week maintenance phase.

The LATTE-2 Phase IIb multicentre open-label study recruited 309 individuals, all of whom received oral therapy during the induction

phase of the trial (oral cabotegravir and Kivexa for 20 weeks, with oral rilpivirine being added in the last 4 weeks) in order to assess for potential hypersensitivity and intolerability. Of these, 286 individuals became virologically suppressed, and were then randomly allocated to one of three arms. The first arm (*n*=56) continued oral treatment (cabotegravir and Kivexa), the second received the combination intramuscular injectable (rilpivirine and cabotegravir) every 4 weeks (*n*=115), and the third group received the injectable every 8 weeks (*n*=115). Viral suppression levels remained high in all arms (91% in oral arm, 94% in 4-weekly dosing and 95% in 8-weekly dosing). Serious adverse events were low overall; however, high levels of mild–moderate injection site reactions were reported (93% described injection site pain). Despite this, patient satisfaction in all arms of the trial was high, and was actually greater (90%) in the injectable compared to the oral daily-dosing arm (70%).

LATTE-2 provides good evidence for both the efficacy and the acceptability to patients of injectable agents. If the cabotegravir/rilpivirine and other injectable combinations are further developed, they are likely to have a major impact on patient lifestyle and satisfaction, including allowing patients greater freedom to travel without medications. These long-acting medications may also provide a key tool to help us effectively treat populations including drug users and adolescents, in whom adherence issues remain a major barrier to the provision of effective treatment.

## References

1. Kaplan G, Casoy A, Zummo J. Impact of long-acting injectable antipsychotics on medication adherence and clinical, functional, and economic outcomes of schizophrenia. *Patient Prefer Adherence* 2013; **7**: 1171–1180.

2. Margolis DA, González-García J, Stellbrink HJ *et al.* Cabotegravir+rilpivirine as long-acting maintenance therapy: LATTE-2 week 32 results. *Conference on Retroviruses and Opportunistic Infections*. February 2016. Boston, MA, USA. Abstract 31LB.

3. Margolis DA, Brinson CC, Smith GH *et al.* Cabotegravir plus rilpivirine, once a day, after induction with cabotegravir plus nucleoside reverse transcriptase inhibitors in antiretroviral-naive adults with HIV-1 infection (LATTE): a randomised, phase 2b, dose-ranging trial. *Lancet Infect Dis* 2015; **15**: 1145–1155.

## FDG PET/CT imaging of lymph nodes as a measure of the HIV reservoir in humans

Jasmini Alagaratnam

*Clinical Trials Unit, St Mary's Hospital, Imperial College London, UK*

Lymphoid structures have been identified as a major HIV reservoir, with evidence of ongoing viral replication despite suppressive antiretroviral therapy (ART) [1]. Hsue *et al.* assessed the association between axillary lymph node (LN) inflammation (using 18F-fluorodeoxyglucose (FDG) PET/CT imaging) and size of HIV reservoir [2]. Tissue with high glucose metabolism (e.g. in inflammation) show a preferential uptake of FDG (an analogue of glucose) [3,4].

This prospective observational study recruited 73 individuals; 44 HIV-infected individuals of whom 34 were suppressed on ART, seven elite controllers, three not on ART and 29 matched HIV-uninfected controls. FDG uptake (as a proxy for inflammation) in the axillary LN was assessed as a mean standardised uptake value (SUV). A target to background ratio (TBR) for LN was generated by dividing LN SUV by blood background SUV. Frequency of cells harbouring integrated HIV DNA in CD4+ T cells measured viral persistence.

FDG PET/CT uptake was higher among HIV-infected individuals on ART and elite controllers compared to HIV-uninfected individuals. Higher uptake of FDG was associated with higher plasma HIV RNA load (r=0.556, $P<0.001$), despite correcting for ART, CD4 count and history of opportunistic infections ($P=0.006$). Additionally, higher LN FDG uptake was associated with viral persistence among elite controllers (integrated HIV DNA in peripheral blood mononuclear cells, r=0.85, $P=0.015$), but not in

those whose HIV was suppressed on treatment. Finally, higher LN FDG uptake was associated with higher markers of CD4+ T cell activation in blood (HLADR+CD38+ cells: r=0.434, *P*=0.005) and higher levels of plasma D-dimer (r=0.32, *P*=0.044).

These findings suggest that HIV production (and perhaps replication) contributes to inflammation and/or a chronic inflammatory environment in lymphoid tissues that may contribute to persistence. FDG-PET/CT imaging of axillary LNs may provide a method to non-invasively measure inflammation and assess responses to different ART or anti-inflammatory interventions.

## References

1. Lorenzo-Redondo R, Fryer HR, Bedford T *et al.* Persistent HIV-1 replication maintains the tissue reservoir during therapy. *Nature* 2016; **530**: 51–56.

2. Hsue P, Takx R, Ishai A *et al.* FDG PET/CT imaging of lymph nodes as a measure of the HIV reservoir in human. *Conference on Retroviruses and Opportunistic Infections.* February 2016. Boston, MA, USA. Abstract 343.

3. Zanoni L, Al-Sugair AS, Fanti S. Clinical applications of 18F fluoro-2-deoxy-D-glucose (FDG) positron emission tomography – computed tomography (PET/CT). *Nuclear Med Rev* 2012; **15 Suppl C**: C48–C51.

4. Vaidyanathan S, Patel CN, Scarsbrook AF, Chowdhury FU. FDG PET/CT in infection and inflammation and current and emerging clinical applications. *Clin Radiol* 2015; **70**: 787–800.

BOOK REVIEW

# ABC of domestic and sexual violence

Angelina Namiba*

Salamander Trust Associate

Bewley S and Welch J (Editors)
*ABC of Domestic and Sexual Violence,* 2014 is available from Wiley, priced £21.99.
ISBN 978-1-118-48218-6

*1 in 3 women worldwide have experienced physical or sexual violence – mostly by an intimate partner* [1]. It is one of the most widespread abuses of human rights worldwide.

Aimed at healthcare professionals, this book is a very timely guide. Particularly so because, due to the intimate nature of the relationships they have with their patients, healthcare professionals are in a unique position to identify the issue, empathise with their patients and refer them for support. The book aims to provide practical help to learners early in their careers. It starts with a foreword by Sir George Alberti of the King's College NHS Trust and also has a concise introduction by the editors.

Chapter authors are experts in the various fields of domestic and sexual violence. They cover: the epidemiology of gender-based violence; culture and violence; domestic violence and abuse; the impact of trauma; children; sexual assault of men and boys; identifying domestic violence and abuse; community-based responses to domestic violence; sources of referral and support for domestic violence; perpetrators; general practice; emergency medicine and surgical procedures; elder abuse; the dental team; mental health services; women's reproductive and sexual health services; female genital mutilation; sexual violence; what to consider; rape and sexual assault; medical and psychosocial care; documenting in the notes; law and prosecuting practice in relation to serious sexual assaults and domestic violence; writing a statement as a professional witness; going to court; violation of professional boundaries; and moving forward: developing care pathways within the health service; and pursuing a career and implementing better services.

Although it covers an extensive area, the style and layout of the book make it easy for the reader to go directly to the relevant topic or area of specialty. This is particularly so as the chapters are short, clear, to the point and very practical. The use of illustrations, question-and-response style and case studies, as well as practical tips on assessment, examination, management and referral of cases, make it a comprehensive and valuable guide.

The only small weakness of the book, from my perspective, is the very basic coverage of HIV. There are sections covering testing and post-exposure prophylaxis (PEP), and a small section covering gender-based violence and links to HIV. Perhaps the authors could consider including a chapter expanding on gender-based violence, and the links to HIV, in a future edition.

All in all, this book is very well researched, expertly written and engaging. In my opinion, it is the definitive guide. I would recommend it highly to healthcare (and other) professionals in the various fields, whether well established or just starting out in their careers.

## Reference

1. UN WOMEN. *Prevent violence against women: The Issue.* Available at: www.unwomen.org/en/news/in-focus/endviolence- against-women (accessed November 2015).

*Correspondence author:* Angelina Namiba
Email: anamiba15@gmail.com