Treatment interruption and predictors for viraemic control

Jintanat Ananworanich

Treatment interruption is a critical step in identifying biomarkers for viraemic control post treatment interruption. It is the ultimate test for HIV remission [1]. The following studies address markers that are associated with viraemic control and are pertinent to clinical research aimed at achieving HIV remission.

In Abstract 110LB [2] the AIDS Clinical Trials Group (ACTG) conducted an analysis across multiple ACTG studies of participants who underwent treatment interruption. Jonathan Li presented data from 124 participants, 104 of whom started antiretroviral therapy (ART) during chronic HIV infection and 20 of whom started ART during acute HIV infection. The majority of participants lost viral control within 4 weeks of interruption (Table 1). The acutely treated patients had a longer time to viral rebound, and by week 24, 13% still had HIV RNA below 200 copies/mL whereas this was achieved in only 3% of chronically treated patients. Two markers of active HIV reservoir predicted time to viral rebound. These were cell-associated HIV RNA in peripheral blood mononuclear cells (PBMCs) and plasma residual viremia above 1 copy/mL. Interestingly, cell-associated HIV DNA was not associated with viraemic control.

| Table 1. Proportion of virological suppression after treatment interruption |
|-----------------------------|-----------------|-----------------|-----------------|
| HIV RNA (copies/mL)         | Week 4 (%)      | Week 12 (%)     | Week 24 (%)     |
| <1000                       | 37              | 11              | 6               |
| <200                        | 31              | 9               | 6               |

This study highlights the importance of evaluating the active HIV reservoir as possible predictors for viraemic control in future treatment interruption studies.

The SPARTAC study evaluated three ART strategies for acute and recent HIV infection: (1) no therapy (standard of care); (2) immediate ART for 12 weeks followed by treatment interruption; and (3) immediate ART for 48 weeks followed by treatment interruption [3]. The study identified cell-associated HIV DNA as a marker for viraemic control post treatment interruption [4]. In Abstract 111LB, John Frater presented additional investigations of possible markers for viraemic control including HIV-specific T cell responses, soluble inflammatory markers, activated T cells and T cell exhaustion [5]. After adjusting for HIV DNA levels, only T cells that express exhaustion markers (PD–1, Lag–3, Tim–3) were associated with viraemic control. Low frequencies of these cells predicted longer time to viral rebound above 400 copies/mL. PD–1, Lag–3 and Tim–3 are immune checkpoint molecules that regulate T cell activation and homeostasis. This study highlights the need to investigate immunological parameters in the identification of biomarkers for viraemic control.

In Abstract 52 [6], phenotype and anti-HIV capacity of natural killer (NK) cells were investigated in post-treatment controllers (PTC) from the VISCONTI cohort [7] along with four other groups (healthy individuals, elite controllers and individuals who were aviraemic or viraemic on ART). Daniel Scott-Algara presented data showing higher frequencies of NK cells that express certain phenotypes such as KIR2DL2 inhibitory receptor and lower expression of CD69 activation marker to be associated with the PTC status, and these were not observed in elite controllers. Additionally, the PTCs had higher anti-HIV capacity of NK cells illustrated by the higher production of IFN-γ with degranulation and lower p24 antigen in autologous CD4 T cells infected in vitro. This study suggests that NK cells have a role in maintaining viral control after the removal of ART.

In Abstract 354 [8], Felipe Garcia presented data from a dendritic cell vaccine study [9] illustrating that cell-associated HIV DNA is stable even after a period of treatment interruption. When individuals interrupted ART for 3 months followed by re-treatment for 9 months, their HIV DNA levels were similar to levels seen prior to treatment interruption. Moreover, the study showed that three doses of dendritic cell vaccine prevented the rise of HIV DNA at 12 weeks following treatment interruption. The HIV DNA control was likely to be a result of HIV-specific CD8+ T cell responses. This study provides evidence for the lack of increased HIV reservoir size after treatment interruption and resumption of ART.

References
1. Ananworanich J, Fauci AS. HIV cure research: a formidable challenge. J Virus Erad 2015; 1: 1–3.
2. Etemad B, Ahmed H, Kuritzkes D et al. The size of the active HIV reservoir predicts viral rebound. Conference on Retroviruses and Opportunistic Infections 2015. February 2015, Seattle, Washington, USA. Abstract 110LB.
3. SPARTAC Investigators, Fedler S, Porter K et al. Short-course antiretroviral therapy in primary HIV infection. N Engl J Med 2013; 368: 207–217.
4. Williams JP, Hurst J, Stohr W et al. HIV-1 DNA predicts disease progression and post-treatment virological control. Elife 2014; 3: e03821.
5. Hurst J, Williams J, Pace M et al. Biomarkers to predict viral rebound at antiretroviral therapy interruption in SPARTAC. Conference on Retroviruses and Opportunistic Infections 2015. February 2015, Seattle, Washington, USA. Abstract 111LB.
6. Scott-Algara D, Didier C, Arnold V et al. Post-treatment controllers have particular NK cells with high anti-HIV capacity: VISCONTI Study. Conference on Retroviruses and Opportunistic Infections 2015. February 2015, Seattle, Washington, USA. Abstract 52.
7. Saez-Cion A, Bacchus C, HoqueLoux L et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLoS Pathog 2013; 9: e1003211.
8. Andres C, Alvarez-Fernandez C, Climent N et al. Viral reservoir dynamics after therapeutic vaccination and cART interruption. Conference on Retroviruses and Opportunistic Infections 2015. February 2015, Seattle, Washington, USA. Abstract 354.
9. Garcia F, Climent N, Guardo AC et al. A dendritic cell-based vaccine elicits T cell responses associated with control of HIV-1 replication. Sci Transl Med 2013; 5: 166ra162.

New insights into HIV persistence, latency reversal and viraemia rebound

Linos Vanderkerckhove

This thought-provoking session, moderated by Françoise Barre-Sinoussi and Celsa A Spina, had many interesting presentations.

For Abstract 104LB [10], Jamal Tazi postulated that either the inhibition or activation of splicing would be detrimental for viral replication. Initially, HIV transcripts are spliced upon transcription to generate the proteins Tat and Rev. Rev inhibits the process of splicing and facilitates the export of HIV mRNA from the nucleus to the cytoplasm. A library of molecules capable of achieving long-lasting effect on viral replication through inhibition of
Rev-mediated viral RNA biogenesis was designed and tested. The library was screened on infected PBMCs from healthy donors and ABX464 was selected as an optimal compound. Sequencing of viral RNA from treated cells established that ABX464 does not select for mutations; however, massive splicing of viral RNA is induced. Using a system to visualize single HIV RNA molecules in living cells, the researchers demonstrated that ABX464 interferes with Rev-mediated export of unspliced HIV-1 transcripts to the cytoplasm. Efficient inhibition of various HIV subtypes in PBMCs and macrophages was demonstrated.

In addition ABX464 alone also efficiently compromised viral proliferation in two humanised mouse models infected with HIV. Of note, there was no rebound of viral load for 2 months following treatment cessation of ABX464 whereas viral load increased dramatically just 1 week after ART treatment. A Phase I study conducted in healthy volunteers has demonstrated that a single administration of ABX464 was well tolerated and a Phase II study has been initiated. ABX464 represents a novel class of anti-HIV molecules. It appears to have a long-lasting effect in humanised mice and is therefore a promising strategy towards HIV cure.

In Abstract 108, another strategy for reactivating latent reservoirs was presented by James Whitney [11]. In a study on SIV-infected rhesus macaques on ART, an oral toll-like receptor 7 (TLR7) agonist was administered to determine whether it would induce transient plasma viraemia and thus reduce viral reservoirs. Ten macaques were infected with SIVmac251 by rectal challenge. Plasma SIV RNA levels were measured by RT-PCR (limit of detection 50 copies/mL). The macaques received ART at ~9 weeks after infection (PI) and became virologically suppressed by 24 weeks following. Virological suppression was maintained through to week 45. Four macaques were then administered seven doses of the TLR7 agonist at twice-monthly intervals, while still on ART. The first three doses of TLR7 agonist had limited effect on plasma viraemia. However, doses 4–7 led to transient and consistent increases in plasma virus (500–1000 SIV RNA copies/mL) in all treated macaques with a return to <50 copies/mL within 4–7 days of TLR7 dosing. After completion of the TLR7 regimen, SIV DNA levels were reduced by 56–75% in PBMC, colon and lymphoid tissues. Viral DNA levels remained unchanged in the placebo control macaques. ART was discontinued to determine whether these transient plasma virus blips and decreases in viral DNA content also reduced the size of the viral reservoir. While the plasma virus rebound kinetics in animals dosed with the TLR7 agonist were comparable to the placebo group after discontinuation of ART, the TLR7-treated animals showed a -0.5 log10 reduction in plasma virus set-point compared to the placebo group.

The authors concluded that these novel results support clinical investigation of a TLR7 agonist in HIV-1-infected patients on ART.

If there is shock but no kill: HIV-1-infected CD4+ T cells can be clonally expanded in response to antigen stimuli and latency-reversing agents

Ya-Chi Ho

Antigen-specific activation may lead to clonal expansion of memory CD4+ T cells containing replication-competent HIV-1. When a naive CD4+ T cell encounters an antigen, it is activated and expanded. It then moves into a quiescent state and becomes a resting memory CD4+ T cell. The cognate antigen is the ‘key’ to specifically activate this memory CD4+ T cell from its resting state. HIV-1 may infect the CD4+ T cells latently as the memory cell enters the quiescent state. What would happen if a cognate antigen activated the resting memory CD4+ T cell containing a replication-competent HIV-1 provirus?

Francesco Simonetti and colleagues reported clonal expansion of HIV-1–infected, tumour-specific CD4+ T cells causing residual viraemia [12]. In this particular patient with squamous cell carcinoma, a population of HIV-1–infected CD4+ T cells, which were found throughout different anatomical sites (including peripheral blood, lymph nodes and tumour infiltrations) were shown to be expanded from the same clone (based on the same HIV-1 integration site). This clone expanded during the course of tumour progression and caused a residual viraemia of one predominant plasma clone (based on the HIV-1 p6 reverse transcriptase sequence). After reconstruction, this clone was found to be replication competent. This implies that a clone of memory CD4+ T cells may respond to tumour antigen stimuli, expand throughout different sites of the body, and cause viraemia.

This specific case demonstrates how a ‘physiological shock’ (tumour antigen) can activate clones of HIV-1–infected cells. The effect of such physiological shock by cognate antigen stimulation is very robust (as shown by residual viraemia) and systemic (as shown by the same clone found in different anatomical sites). Although new rounds of infection are presumably blocked by effective concurrent antiretroviral therapy, such shock exponentially expanded the size of the latent reservoir by increasing the numbers of cells containing this replication-competent HIV-1. Therefore, ‘shock’ without an effective ‘kill’ may paradoxically increase the size of the latent reservoir.

The shock-and-kill strategy may also induce clonal expansion of the latent reservoir. Unlike cognate antigen stimulation, the shock-and-kill strategy presumably reactsivates HIV-1 without T cell activation. Unlike antigen stimulation, which leads to T cell proliferation, theoretically, latency-reversing agents would not expand the size of the latent reservoir through clonal expansion of HIV-1–infected resting CD4+ T cells.

Kirston Barton et al. examined HIV-1 DNA, cell-associated HIV-1 RNA and plasma HIV-1 RNA in a shock-and-kill trial using the histone deacetylase inhibitor, panobinostat [13]. Although panobinostat reactivated non-selectively in most patients, one patient showed clonal expansion of HIV-1 DNA and cell-associated RNA, which led to viral rebound during treatment interruption (based on single genome sequencing of the env region).

In summary, it is critical to enhance the ‘kill’ part of the shock-and-kill strategy. If there is only shock but no kill, the size of the HIV-1 latent reservoir may potentially increase through clonal expansion.
markers can be helpful in evaluating the efficacy of therapies that shorter time to viral rebound [2], concluding that these viral the time of ART interruption were significantly associated with a cell-associated RNA levels and higher residual viraemia values at Jonathan Li and colleagues showed that higher HIV-1 when antiviral drugs are no longer present, highlights the Moreover, the existence of an HIV-1 ‘reservoir’ capable of translocation and fibrosis remain elevated in acute HIV-1-infected individuals despite early ART initiation [15].

The role of latency as a major barrier to finding a cure for HIV Claudia Alteri, Francesca Ceccherini Silberstein and Carlo-Federico Perno

Achievement of a ‘functional cure’ for people living with HIV still seems to be far off in the light of recent setbacks [14]. Although current antiretroviral therapy suppresses HIV replication and halts the otherwise inevitable progression to AIDS, ART is not curative, and cannot prevent the inflammatory damage caused by HIV. In this regard, results from Abstract 47, presented by Netanya Utay and colleagues, showed that inflammation, microbial translocation and fibrosis remain elevated in acute HIV-1-infected individuals despite early ART initiation [15].

Moreover, the existence of an HIV-1 ‘reservoir’ capable of producing infectious virus that can re-establish active infection, when antiviral drugs are no longer present, highlights the importance of developing ‘curative’ strategies. In this regard, Jonathan Li and colleagues showed that higher HIV-1 cell-associated RNA levels and higher residual viraemia values at the time of ART interruption were significantly associated with a shorter time to viral rebound [2], concluding that these viral markers can be helpful in evaluating the efficacy of therapies that aim to achieve sustained ART-free HIV remission. Along the same lines, John Frater and colleagues, presenting results from the SPARTAC study, found that by analysing immunological and virological biomarkers in 47 individuals undergoing treatment interruption, the expression of PD-1, Lag3, Tim-3, and amount of total HIV-1 DNA, can help to predict time to HIV rebound in patients in treatment interruption [5].

However, has CROI 2015 improved knowledge about the source of this residual viraemia, about the nature and size of the HIV-1 reservoir? Has CROI 2015 clarified the role of ART in the reduction of reservoir size and in preventing return of the virus after cessation of therapy?

It is important to bear in mind that the true active HIV-1 reservoir during therapy is a very small fraction of the total HIV-infected cells, which are stably present in individuals on therapy. The majority of these cells harbour defective virus, often containing large internal deletions or APOBEC-mediated G-to-A hypermutations. For these reasons, characterising the proviral strains able to produce a replication-competent virus, and defining the producer cells, is, to date, essential.

However, even if the HIV-1 genome contains lethal mutations, the LTR promoter may remain intact, indicating that HIV-1 RNA may still be transcribed. In line with this, the study presented by Ya-Chi Ho showed that defective HIV-1 proviruses may be transcribed during latency reversal and that cells containing defective HIV-1 proviruses may expand under T cell activation [16]. Thus, the transcription of HIV-1 RNA from defective proviruses may complicate the measurement of the size of the latent reservoir and its role in the pathogenesis, since defective proviral RNA does not indicate the reactivation of the clinically significant replication-competent proviruses.

To establish whether HIV-1 expression during ART results from spontaneous reactivation from latency or from continuous low-level virus transcription, two studies were presented by Mary Kearney and Marta Bull, respectively [17,18]. Both studies compared HIV-1 cell-associated RNA with HIV-1 cell-associated DNA and HIV-1 plasma RNA by single genome assay. Both studies confirmed that low-level viraemia, or blips, often arise from proliferating cells induced to transcribe proviral sequences, and that an ongoing viral evolution, and a continuing viral replication can be observed despite suppressive therapy. Bull et al. [18] also reported that in several cases the presence of ‘defective’ proviral strains can produce viraemia without infection of additional cells, thus confirming the data presented by Ya-Chi Ho [16].

Francesco Simonetti and colleagues [12] reported a clinical case in which residual viraemia was associated with an expanded cell line carrying a specific intact HIV provirus. By recovering 317 HIV sequences from plasma, peripheral blood mononuclear cells, spleen, lymph nodes and tumour tissues, which were infiltrated with both CD4+ and CD8+ T cells, they found that HIV variants were well mixed across blood and tissues, and there was no evidence of localised replication. A single provirus was the cause of the majority of the HIV RNA detected in plasma. Cells from this clone accumulated specifically in cancer metastases, suggesting that immune stimuli, like tumour antigens, can contribute to cell expansion, and perhaps to the activation of the provirus and release of virions into plasma.

Finally, it is known that HIV establishes a latent reservoir in resting CD4 cells in the earliest weeks of primary infection. To determine the impact of early ART on HIV DNA decay in humans, Moussa Laanani and colleagues [19] made 1305 HIV DNA measurements from peripheral blood mononuclear cells in 327 people enrolled in the PRIMO cohort during primary infection, who began ART within the month of enrolment, and reached a plasma viral load below 50 HIV RNA copies/mL within 6 months. Laanani and colleagues calculated that HIV DNA declined fastest in the first 8 months of ART among cohort members who started ART within 15 days of infection [19]. HIV DNA decay rates were slower in people who started ART within 1 month of infection and slower still in those who started within 3 months of infection. Moreover, average intracellular HIV DNA after 5 years of uninterrupted suppressive ART would be smaller (1.62 log_{10} copies/million PBMCs) in the group that started ART within 15 days of infection than in the group that started ART within 3 months of infection (2.24 log_{10} copies/million PBMCs; P = 0.0006) [19]. Overall these data provided strong arguments in favour of ART initiation at the earliest possible time point after HIV infection, and thus are in favour of early screening.

In conclusion, most of the research presented at CROI 2015 deepened our understanding of the persistence of HIV latent reservoirs in the body, even among ART-treated individuals. While there is a long way to go before reaching clinically relevant results, all these studies are valuable since they suggest possible new approaches to target residual virus, and decrease the reservoir to below a clinically relevant threshold.

References

12. Simonetti F, Hill S, Wu X et al. Residual viremia caused by clonally expanded tumor-infiltrating CD4+ cells. Conference on Retroviruses and Opportunistic Infections 2015. February 2015. Seattle, Washington, USA. Abstract 105.

13. Barton K, Hiener B, Palmer S et al. Panobinostat broadly activates latent HIV-1 proviruses. Conference on Retroviruses and Opportunistic Infections 2015. February 2015. Seattle, Washington, USA. Abstract 109.
Young investigators unravel persistent HIV RNA transcription at CROI 2015

Ward De Spiegelare

At CROI 2015, a panel of young investigators provided new insights into the transcription of HIV RNA within the viral reservoir. This persistent viral reservoir, composed of integrated HIV DNA, which can produce new replication-competent virus, forms the major hurdle towards an HIV cure. It is still unclear if this reservoir is truly dormant or if it is characterised by low-level transcription and ongoing virus production. Initial data indicate that free plasma virus can be found within the blood of patients on cART, and HIV DNA transcription has been observed by several studies.

A study presented by Christopher Pohlmeier [20], showed that HIV RNA is transcribed in HIV-infected patients on combination antiretroviral therapy (cART) and also, to a lesser extent, in elite controllers who can suppress HIV without cART. Detection of viral RNA transcripts in cells may be biased by readthrough transcripts, which are transcribed due to upstream promoter of genes in which HIV integrates, and not due to the viral promoter itself. However, Alexander Pasternak [21] showed that readthrough transcripts form only a minor fraction of the total HIV RNA recorded in cells of HIV-infected patients on cART (less than 8% at best). This shows that the detected cell-associated viral RNA transcripts are most likely to be a product of the HIV promotor-driven transcription. Interestingly, latently infected cells may also produce HIV proteins without producing virus. This was shown by Laura DeMaster [22] in a study on in vitro latently infected cells in which expression of Gag proteins and Nef-associated downregulation of CD4 was observed.

Feiyu Hong [23] revealed that this ongoing transcription is correlated to the total pool of integrated HIV DNA, but not the level of residual viraemia in the blood, suggesting that cellular HIV RNA transcription in HIV-infected patients on cART does not necessarily lead to viral production. The correlation with total viral DNA is striking, as only a minor pool of the total HIV DNA represents the viral reservoir which is composed of intact replication-competent HIV DNA. However, the correlation of HIV DNA with cell associated viral RNA may be explained by the findings of Ya-Chi Ho [16] who revealed that viral transcripts can be transcribed from defective integrated sequences. Interestingly, she also showed that cells, producing HIV RNA from defective HIV sequences in vitro can be killed by a CTL response. Finally, Christina Yek [24] found that vaccination against non-HIV pathogens (Influenza, Pneumococcus) can induce a transient increase of HIV RNA transcription, but does not lead to a reduction of the viral reservoir as assessed by HIV DNA.

In conclusion, this panel revealed interesting findings, which may be useful in the pursuit of an HIV cure. The finding that HIV RNA, and maybe even viral protein, is expressed in the viral reservoir shows that the HIV reservoir is not completely silent. However, HIV RNA levels can be biased by RNA transcripts, originating from defective viral integrants. Current efforts to shock and kill the HIV reservoir have been successful at increasing viral RNA transcription, but the pool of HIV DNA is not effectively purged by transcriptional activation only.

References

16. Ho Y-C, Pollack R, Yong P, Siliciano R. Defective HIV-1 proviruses can be transcribed upon activation. Conference on Retroviruses and Opportunistic Infections 2015. Seattle, Washington, USA. Abstract 105.

20. Pohlmeier C, Bullen CK, Laird G et al. Measurements of viral transcription in elite suppressor CD4+ T cells. Conference on Retroviruses and Opportunistic Infections 2015. February 2015. Seattle, Washington, USA. Abstract 427.

Yek C, Gianella S, Massanella M. Influenza vaccination increases HIV-1 transcription during antiretroviral therapy. Conference on Retroviruses and Opportunistic Infections 2015. February 2015. Seattle, Washington, USA. Abstract 391.

Imaging HIV reservoirs and antiretroviral drug biodistribution: a beacon of hope for HIV cure

Esther Cathgoth

CROI is a highly anticipated forum that draws on novice to expert researchers from across the globe with a focus on HIV scientific research. In this short report, we highlight some of the new innovative techniques in imaging HIV reservoirs and drug biodistribution that were reported at CROI 2015.

Treatment of HIV infection with antiretroviral drug therapy has allowed for sustained control of viral replication; however, latent reservoirs prevent virus eradication. The establishment of HIV reservoirs occurs throughout the body. This widespread latent viral dissemination coupled with heterogeneous distribution of antiretroviral agents is a major obstacle in achieving complete HIV cure. Bioimaging offers an inventive avenue for researchers to understand viral behaviour and drug distribution on a micro- and macro-level. Imaging instruments have been evolving over the years to provide the researcher with more detailed and accurate biological information, and with better resolution. This advancement offers great opportunities for basic science and drug development research to achieve HIV cure, remission or vaccine. At CROI 2015, a promising novel technology for imaging the spatial distribution of antiretrovirals was presented.

Elias Rosen presented an animal study in rhesus macaques of the use of infrared matrix-assisted laser desorption electrospray ionisation (IR-MALDESI) source coupled to a Thermo Q-Exactive mass spectrometer to image (MSI) the concentration of efavirenz in HIV-1 viral reservoir tissues [25]. The tissue reservoirs studied included gut-associated lymphoid tissue (GALT), lymph nodes, brain and testes. Efavirenz concentrations were determined at steady state for a once-daily 200-mg dose administration. Imaging required 10 μm cryosections of snap-frozen tissue for analysis. Quantitation throughout the entirety of the tissue was performed against matching tissue controls not containing drug. Images confirmed the presence of efavirenz in all the tissue...
samples and total exposure ranged by a factor of 20 from the testes to the colon. This trend was confirmed by serial liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses. This demonstrates the heterogeneous distribution of efavirenz in the different tissues. Authors combined the IRMALDESI MSI results with imaging tissue morphology and immunohistochemistry to reveal information about where efavirenz was concentrated. For example, colon imaging showed efavirenz concentrated in the mucosa and lamina propria of the colorectal epithelium, which corresponded to regions of high CD3+ T cell density. No such mucosal enhancement was observed in the ileum. In the brain tissue, there was enhanced efavirenz exposure in the grey matter relative to white matter and with concentrations lowest in the basal ganglia (167 pg/g tissue). This concentration increased by approximately two-fold in most other tissues (cerebrum, lymph nodes, spleen, testes and most GALT) with highest concentrations in the rectal tissue (3.6-fold). The authors also found significantly higher concentrations in tissue compared to plasma. In particular, efavirenz concentration in CNS tissue was 6 log10 higher and for the remaining tissues and 2 log10 higher than plasma. These preliminary results indicate great potential for determination of antiretroviral tissue penetration specific to sites of action and identification of target therapeutic antiretroviral concentrations. This technique is currently being tested on 10 critical antiretroviral drugs with an aim to image all antiretrovirals available on the market.

Reference
25. Rosen E, Bokhart M, Muddiman D et al. Imaging the spatial distribution of efavirenz in intact HIV tissue reservoirs. Conference on Retroviruses and Opportunistic Infections 2015. February 2015. Seattle, Washington, USA. Abstract S35.

New antiretroviral agents for HIV
Alessandro Cozzi-Lepri

Perhaps the most interesting stories regarding new HIV drugs out of CROI 2015 are the development of two new HIV therapies – the new attachment inhibitor (BMS-663068) and the new maturation inhibitor (BMS-955176), as well as the data on safety and efficacy of tenofovir alafenamide (TAF). Although the prevalence of HIV-infected people with extensive drug resistance to all currently available drug classes in the clinics seems to be low and stable, drugs that work in novel ways could be particularly beneficial for highly treatment-experienced people who might develop extensively resistant virus in the near future.

Tenofovir alafenamide (TAF)

David Wohl from the University of North Carolina presented combined results looking at the antiviral activity of a new TAF single-tablet regimen (Abstract 113LB) [26]. In the studies, 1073 antiretroviral-naive adults were randomised to start either a TAF- (n=866) or TDF-based (n=867) combination treatment in two identical international Phase III trials, each as part of a once-daily formulation also containing elvitegravir, cobicistat and emtricitabine. It was planned in the protocol that results of these two double-blind, double-dummy trials for the 48-week analysis would be pooled. All study participants had an estimated glomerular filtration rate (eGFR) ≥50 mL/min at baseline, and none had hepatitis B or C. Paul Sax from Brigham and Women’s Hospital in Boston followed with data on TAF’s effects on kidneys, bones, and lipids (Abstract 143LB) [27].

Abstract 113LB [26] aimed at establishing the virological non-inferiority of the TAF formulation compared to the TDF formulation. The primary endpoint was the proportion of people with a viral load (VL) ≤50 copies/mL at 48 weeks using the FDA snapshot endpoint. Median ages of the two groups were 33 and 35 years, 85% in both groups were men, about 25% black and 19% Hispanic/Latino. Median pre-ART CD4 cell count was 404 cells/mm3 in the TAF arm and 406 cells/mm3 in the TDF arm. Pre-ART viral load was 4.58 log10 copies/mL in both arms. The two arms seemed balanced for all measured characteristics.

By 48 weeks from enrolment, 5% stopped treatment in the TAF arm and 8% stopped in the TDF arm. At 48 weeks 92% in the TAF arm and 90% in the TDF arm had a VL ≤50 copies/mL. The difference between arms (2.0%, 95% CI: -0.7%–4.7%) and therefore virological non-inferiority of TAF to TDF in this single-tablet combination given to previously untreated people was established. Resistance profiles for people whose treatment failed also appeared to be similar. Virological response rates did not differ between study arms after stratifying participants by pre-treatment viral load above or below 100,000 copies/mL, baseline CD4 cell count above or below 200 cells/mm3, age above or below 50 years, gender or race (no significant interactions detected). Treatment was generally safe and well tolerated, with no statistical differences in overall drug safety profiles between arms. There were few serious adverse events (8% for TAF vs 7% for TDF) or drug discontinuation for this reason (0.9% for TAF vs 1.5% for TDF) in either group. The most common side effects were diarrhoea (18%), nausea (16%) and headache, all occurring with similar frequency in both groups.

Although it was reassuring to see that virological non-inferiority and general safety was established it remained unclear from this first presentation whether TAF was indeed inducing less renal toxicity than TDF, which was the main objective for conducting a non-inferiority trial.

These potential concerns were partially addressed in the following Abstract 143LB [27] by Paul Sax and colleagues. In this analysis, pre-specified safety endpoints were serum creatinine, proteinuria, and hip and spine bone mineral density (BMD). Tenofovir levels in plasma were 91% lower with TAF than TDF. But tenofovir levels in cells were four times higher with TAF. At week 48 serum creatinine increased on average by 0.11 mg/dL with TDF versus 0.08 mg/dL with TAF (P<0.001), consequently eGFR fell by an average of 11.2 mL/min with TDF versus 6.6 mL/min with TAF (P<0.001). None of the participants who received TAF and four people receiving TDF (0.5%) left the study because of renal adverse events (two renal failures, one decreased eGFR, one nephropathy). Three people in the TAF arm (0.3%) and four in the TDF arm (0.5%) had hypophosphataemia, and two people in each arm (0.2%) had a grade 2 or greater increase in proteinuria. Nobody in the trial developed proximal tubulopathy. Healthier values of urine protein, albumin and β2-microglobulin through 48 weeks were observed in the TAF compared to the TDF arm (P<0.001). Overall, there was a general sense that TAF might cause less toxicity than TDF. However, despite highly statistical significant differences for some of the parameters the clinical difference appeared to be small and it remains unclear whether the small improvement in safety profile justifies the much higher cost of the new compound (TDF is likely to become generic in the near future).

Maturation inhibitor BMS-955176

Max Lataillade from Bristol-Myers Squibb presented findings from a Phase IIa proof-of-concept study evaluating BMS-955176, a second-generation maturation inhibitor (Abstract 114LB) [28]. Maturation inhibitors interfere with protease cleavage of the Gag polyprotein, leading to the release of immature virus particles that cannot complete their lifecycle.
and are not infectious. The development of the first compound in this class (bevirimat) was stopped in 2010. BMS-955176 appears to be a more promising drug. It has a long half-life in the body, is suitable for co-formulation and remains active against HIV with the Gag polymorphisms that caused bevirimat to fail. The analysis presented at CROI, conducted in Germany, enrolled 60 previously untreated participants with HIV subtype B. Median age was about 37 years, and the median CD4 cell count was approximately 500 cells/mm³. Participants were randomly assigned to receive BMS-955176 monotherapy at doses of 5, 10, 20, 40, 80 or 120 mg, or else placebo, once-daily for 10 days. They were then observed off treatment for an additional 14 days. BMS-955176 produced maximum median declines in HIV viral load ranging from 0.49 log₁₀ copies/mL in the 5-mg arm to 1.70 log₁₀ copies/mL in the 40-mg arm. The majority of participants still had viral load at least 1 log₁₀ below their baseline level a week after the end of treatment, likely to be due to the drug’s long half-life. BMS-955176 was generally safe and well tolerated at all doses tested. There were no deaths, serious adverse events, study discontinuations due to adverse events or clinically relevant laboratory abnormalities. Four people taking BMS-955176 developed mild-to-moderate diarrhoea. Based on these findings, a Phase Ib study of BMS-955176 is expected to start in the second quarter of 2015. A limitation of the study is that all participants were men, so that results are not generalisable to women.

**Attachment inhibitor BMS-955176**

Combination antiretroviral therapy (ART) consists of drugs that target different steps of the HIV lifecycle. None of the currently approved agents blocks the very first step, initial attachment of the virus to a host cell. BMS-663068 is a pro-drug or precursor of BMS-626529, which binds directly to the gp120 protein that makes up part of the ‘spikes’ on HIV’s outer surface, thereby preventing viral attachment and entry into CD4 T cells. CCR5 blockers like maraviroc and fusion inhibitors like enfuvirtide work at slightly later steps; BMS-663068 is active regardless of whether an HIV strain uses CCR5 or CXCR4 co-receptors. Melanie Thompson from the AIDS Research Consortium of Atlanta and colleagues conducted a Phase Ib trial (AH438011/NCT01384734) to investigate the safety, efficacy, and dose-response characteristics of BMS-663068 in treatment-experienced people with HIV [29]. This study included 254 randomised participants. With sites in South Africa and other middle-income countries, it had a higher proportion of women and non-white people than many antiretroviral drug trials. A majority (60%) were men, just over 30% were white, 30% were black, and the median age was 39 years. Two-thirds had HIV subtype B. At study entry participants had HIV viral load of at least 1000 copies/mL, with about 40% having high viral loads above 100,000 copies/mL. Overall they had relatively advanced disease, with a mean CD4 cell count of approximately 230 cells/mm³ and nearly 40% having less than 200 cells/mm³. Many participants had failed first- or second-line ART and about half had at least one major mutation conferring resistance to at least one widely used antiretroviral drug class. They were, however, required to still be sensitive to raltegravir, tenofovir and the comparator drug atazanavir. Pre-treatment phenotypic screening was performed to ensure that their HIV was likely to be susceptible to BMS-626529. Study participants were randomly allocated to five treatment arms. The first four groups received BMS-663068 at doses of 400 mg or 800 mg twice daily, or 600 mg or 1200 mg once daily, while a control group received ritonavir-boosted atazanavir. Everyone also took tenofovir and raltegravir. Week 48 results were presented at the Conference. There was no evidence for a difference in virological and immunological response rates comparing BMS-663068 and atazanavir/r arms through week 48 and all BMS-663068 doses were generally well tolerated with no dose-response safety signals reported. As a potential limitation, participants receiving BMS-663068 had a higher daily pill burden than those taking atazanavir/r, which could have an effect on adherence. In addition, there were missing data for resistance as the phenotypic assay used to determine BMS-663068 susceptibility was not able to provide results for about one-quarter of participants. Given these promising results, a Phase III clinical trial of BMS-663068 has been planned.

**References**

26. Wohl D, Pozniak A, Thompson M. Tenofovir alafenamide (TAF) in a single-tablet regimen in initial HIV-1 therapy. *Conference on Retroviruses and Opportunistic Infections 2015*. February 2015. Seattle, Washington, USA. Abstract 113LB.

27. Sax PE, Saag MS, Yin MT et al. Renal and bone safety of tenofovir alafenamide vs tenofovir disoproxil fumarate. *Conference on Retroviruses and Opportunistic Infections 2015*. February 2015. Seattle, Washington, USA. Abstract 143LB.

28. Hwang C, Sevinsky H, Ravindran P et al. Antiviral activity/safety of a second-generation HIV-1 maturation inhibitor. *Conference on Retroviruses and Opportunistic Infections 2015*. Seattle, Washington, USA. Abstract 114LB.

29. Thompson M, Lalezari J, Kaplan R et al. Attachment inhibitor produg BMS-663068 in ARV-experienced subjects: week 48 analysis. *Conference on Retroviruses and Opportunistic Infections 2015*. February 2015. Seattle, Washington, USA. Abstract 545.