Highlights of the 9th edition of the Conference on HIV Persistence During Therapy, 10–13 December 2019, Miami, USA

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Introduction

The *Conference on HIV Persistence During Therapy* provides an international forum in which researchers present the most recent advances in HIV cure research. The conference, which now represents the largest gathering of researchers focused on HIV cure research, alternates between Miami and the National Institutes of Health (NIH) in Washington DC. The 9th edition was held in Miami, 10–13 December 2019 and attempted to cover most aspects of HIV cure research from basic mechanisms of HIV latency (Session 1), to new therapeutic approaches with which to eliminate viral reservoirs (Sessions 6 and 8).

NIMH satellite symposium: CNS and myeloid cell reservoirs

The conference also hosted a satellite session, supported by the National Institute of Mental Health (NIMH), that predominantly featured research on HIV persistence in myeloid cells. This is a poorly understood and contentious area and some of the presentations in the satellite symposium focused on addressing whether HIV-1 is able to persist in myeloid-lineage cells. There are several challenges to assessing whether HIV-1 can persist in these cells, which are a highly heterogenous population of cells that reside in almost all tissues; some that are anatomically difficult to sample in living human trial participants. Therefore, investigators have used the non-human primate (NHP) model to enable more comprehensive sampling of anatomical sites where infected macrophages might persist. Thomas Hope (Northwerstern, Chicago, USA) has been using the NHP model to determine the sources of virus that rebound following analytic treatment interruption. His group used a combination of positron emission tomography and computed tomography (PET-CT) to visualise the distribution of virus in tissues using radioactive and bioluminescent probes. The analysis surprisingly revealed that following treatment interruption of monkeys that had been infected for >6 months, viral signals appeared in the heart and the infected cells were identified as macrophages. Dr Hope presented a model in which myeloid cells in the heart may support viral persistence during antiretroviral therapy (ART) and contribute to viral rebound when ART is interrupted. Those studies in NHPs were further enforced by presentations on human clinical trial participants. Viviane Machado (University of Miami, USA) examined post analytic treatment interruption (ATI) viremia for the presence of macrophage-tropic variants. Macrophages express 20-fold less CD4 on their surface than CD4+ T cells. As such, infection of macrophages can only be achieved by viral variants whose envelopes have a high affinity for the CD4 receptor. Ms Machado obtained plasma from individuals undergoing ATI and cloned viral envelopes from plasma

to determine their tropism for macrophages. A low frequency of macrophage-tropic variants were identified in most individuals and some of these variants were highly divergent from their T-tropic counterparts. In addition, use of immunoaffinity enrichment with antibodies to macrophage-specific proteins, as well as molecular clock analysis, enforced the notion that some of these macrophage-tropic variants in plasma directly originated from tissue macrophages and were established during ART. A similar study was presented in Session 5 by James Johnson of the Centers for Disease Control and Prevention (CDC). This group used antibody enrichment approaches to identify viruses in semen that may have originated from macrophages. The approach relies on the principle that during virion budding, the viral particle derives its membrane from the membrane of the host cell. As such, virions derived from macrophages would be expected to contain macrophage-specific markers (such as CD14) on their membrane. Semen from virologically suppressed subjects on integrase-containing regimens had low viral loads that surprisingly, contained virus particles that were predominantly myeloid-cell derived. HIV-1 has previously been demonstrated to reside in urethral macrophages obtained from ART-suppressed individuals undergoing gender reassignment [1] Therefore, it is tempting to speculate that HIV-1 in seminal fluid originates from urethral macrophages that persist in the face of suppressive ART. Collectively these studies provide intriguing evidence for the existence of a myeloid cell reservoir that persists in individuals on suppressive ART. Important questions, such as the longevity of this reservoir and its anatomic distribution, remain to be addressed.

If HIV-1 persists in myeloid cells, there are a number of pressing issues that need to be addressed. Research on CD4+ T cell reservoirs has provided important tools to investigate those reservoirs, such as latency assays, latency reactivation and reservoir elimination strategies. However, many of these tools that have been developed for CD4+ T cell reservoirs are not as well developed for the study of myeloid cell reservoirs. Tim Hanley (University of Utah, Salt Lake City, USA) described attempts to establish HIV-1 latency in primary myeloid cells. The investigators observed that depletion of the cellular transcription factor NF-kB induced a state of viral latency and that viral reactivation could be achieved with agents that have been shown to reverse latency in CD4+ T cells (such as phorbol esters) but not agents such as SAHA. These *in vitro* systems should prove very useful for assessing agents that reverse latency in myeloid cells and help guide strategies for myeloid reservoir elimination. The 'kick and kill' strategy for elimination of latently-infected CD4+ T cells first involves latency reversal with a latency reactivating agent (LRA) so that the infected cell can be cleared by viral cytopathic effects on the host cell and/or immune clearance of the infected cell. Prior studies have suggested that macrophages are resistant to viral cytopathic effects

and to immune clearance by CD8 T cells. Kiera Clayton (Ragon Institute of MGH, MIT and Harvard, Cambridge, USA), extended this issue by demonstrating that infected macrophages are also resistant to NK-cell mediated killing. Similar to that observed for resistance to CD8 T cell killing, infected macrophages also resisted NK-cell killing by presenting an as yet inhibitory signal to NK cells. Furthermore, macrophages appeared less sensitive to killing by NK cells even in the presence of antibody-dependent cellular cytotoxicity (ADCC) which normally enhances NK killing. These results highlight some of the challenges that are likely to arise with strategies that rely on host cell cytopathicity and immune clearance to enable clearance of macrophage reservoirs.

Opening keynote lecture

The opening keynote lecture was given by Anthony Fauci, Director of the National Institute of Allergy and Infectious Diseases (NIAID). He focused on the role that strategies for curing HIV infection might play in reaching the goal of ending the HIV pandemic. While it is possible to control the epidemic by maximally implementing the currently available tools for HIV treatment and prevention, achieving this goal will require overcoming significant barriers related to health disparities and adherence. Likewise, developing strategies to cure HIV infection in the individual will not be sufficient to end the epidemic due to similar implementation challenges. However, by leveraging common scientific advances across HIV treatment, prevention, and cure, it should be possible to develop new and improved tools that can be combined with best practices, identified through implementation science research to control the HIV epidemic (<1 transmission per 10,000 people per year) and, ultimately, lead to elimination within some populations. Towards this goal, NIH is supporting the 'Ending the HIV epidemic: a plan for America' initiative by funding innovative implementation science through its Centers for AIDS Research and AIDS Research Center programmes and has partnered with the Bill & Melinda Gates Foundation (BMGF) on the aspirational goal of developing strategies for *in vivo* delivery of gene-based cures for HIV infection that can be scaled and implemented globally.

In a second opening talk, Mike McCune expanded on BMGF's goal to bring curative interventions for HIV to resource-limited parts of the world through its HIV Frontiers and HIV Reservoirs Consortium programmes and strategic partnerships. He noted the benefits that an HIV cure would have for resource-rich populations (reducing comorbidities, polypharmacy and stigma) as well as in resource-limited parts of the world, where 18 million people living with HIV are not on therapy and modelling predicts that a curative intervention would greatly reduce new infections [2]. Dr McCune outlined the ideal target product profile for an HIV cure as an *in vivo* genetic modification delivered percutaneously in a 'single shot' during a single encounter that lowers viral load to <400 copies/mL without ART for >3 years and prevents or controls new infection. The ideal product will also need to be safe and cost less than US$ 100 per year, including monitoring or predicting loss of remission. Eradication of rebound-competent proviral DNA is not necessarily a requirement to achieve this goal.

Satellite symposium: NIH Martin Delaney Collaboratories

A satellite symposium was convened by NIAID to provide updates on research highlights from each of the six Martin Delaney Collaboratories (MDCs) for HIV cure research. The programme is co-funded by NIAID, the National Institute on Drug Abuse (NIDA), NIMH, and the National Institute of Neurological Disorders and Stroke (NINDS) and is designed to facilitate partnerships between academia, industry, government and community to move HIV cure research forward faster than could be achieved by individual groups working alone.

Steven Deeks outlined the research agenda for the DARE Collaboratory based at the University of California, San Francisco, which aims to: (1) identify the location HIV/SIV persistence in tissues; (2) measure the reservoir; (3) determine the key host immune response characteristics that contribute to persistence; and (4) determine the role of CD8+ T cells in control/elimination of SIV/HIV. Afam Okoye (Oregon Health and Science University, Beaverton, USA) showed that CD8+ T cell depletion in rhesus macaques on 12 months of ART did not delay SIVmac239M rebound but did reduce viral load set point after treatment interruption. Similarly, treatment with anti-PD-1 mAb (nivolumab) did not delay rebound, but significantly reduced viral load set point in a subset of animals.

Luis Montaner from the BEAT-HIV Collaboratory based at Wistar Institute introduced two investigators. Leticia Kuri-Cervantes (University of Pennsylvania, USA) demonstrated that the lymphocyte migration inhibitor, FTY720, causes near complete sequestration of CD4+ T cells in tissues in SIV-infected rhesus macaques on ART but does not affect plasma viremia or viral rebound. She also used thoracic duct cannulation to demonstrate that more HIV can be found in efferent lymph draining from lymphoid tissues than in peripheral blood and that there is no phylogenetic difference between virus in the two compartments. Francesco Simonetti (Johns Hopkins University, Baltimore, USA) used T cell receptor sequencing in conjunction with the Intact Proviral DNA Assay (IPDA) to demonstrate that large numbers of HIV proviral clones persist in CMV-specific CD4+ T cells. Most of these proviruses are defective, however some are replication competent.

Brad Jones presented highlights from the BELIEVE Collaboratory based at Weill Cornell Medicine. He showed data from the IPDA using a bank of clade B samples from ART-treated donors in Canada, Mexico, and USA in which he found that up to 30% of samples gave false negative signals in the standard IPDA and that additional, optimised primers were necessary to accurately detect intact proviruses from some donors. James Whitney (Beth Israel Deaconess Medical Center [BIDMC], Boston, USA) presented results from a study of IL-15 superagonist (N-803 from NantKwest) alone, broadly neutralising antibody (bNAb) 10-1074 alone, or a combination of N-803 + 10-1074 in rhesus macaques infected with SIV/HIV hybrids-AD8 (SHIV-AD8) and suppressed on ART for >1 year. Durable control of viremia after treatment interruption was achieved in 3/5 animals in the combination group. In a second study, N-803 was combined with two bNAbs (10-1074 + 3BNC117), resulting in control of viremia in 6/8 animals.

Dan Barouch provided an overview of the research strategy of the I4C Collaboratory at BIDMC in Boston, which is to pursue both NHP and human clinical studies using combinations of immune stimulants (TLR agonists) with either bNAbs (to achieve rapid elimination of the majority of infected cells) or therapeutic vaccines (Ad26/MVA or dendritic cell-based) to augment immune surveillance and achieve long-term control of the residual reservoir. He introduced two investigators: Po-Ting Liu (BIDMC), who used sequence analysis to show that rebound virus closely matches intact proviruses from both lymph nodes and peripheral blood in 16/16 SIVmac251-infected rhesus macaques following 64 weeks of ART; and Kshitij Wagh (Los Alamos National Laboratory, New Mexico, USA), who discussed why combinations of three bNAbs will likely be required in clinical studies to combat

subtype-specific resistance among different classes of bNAbs and within-host viral sequence diversity.

Hans-Peter Kiem from the defeatHIV Collaboratory at the Fred Hutchinson Cancer Research Center (FHCRC) reviewed his group's approach to using the NHP model to test various combination strategies in a background of HIV-resistant T cell therapy, including chimeric antigen receptor (CAR) T cells, AAV-expressed eCD4-Ig, anti-PD-1 antibody, conserved element DNA vaccination, and TLR7 agonism. Christopher Peterson (FHCRC) outlined his group's comprehensive approach to optimise CAR T manufacture, boost CD4+ CAR T levels, validate the macaque model with a positive control CAR T, and stimulate CAR T proliferation and persistence using a cell-associated Env protein boost. He presented results form a study of CAR T cells in ART-suppressed (> 1 year) SHIV-1157ipd3N4-infected pigtail macaques in which 3/4 animals showed significant delay in viral rebound following treatment interruption, up to 89 days and durable control of viremia in 1/4 animals.

David Margolis outlined the strategy being pursued by the CARE Collaboratory based at the University of North Carolina at Chapel Hill to develop novel anti-latency therapies and combine them with antibody-based immunotherapies to reduce the size of the viral reservoir in both humanised mouse and NHP models as well as in clinical trials in humans. He introduced two investigators. The first, Anne-Marie Turner (University of North Carolina at Chapel Hill, USA), described the development of novel bivalent chemical degraders using proteolysis targeting chimera (PROTAC) technology. She designed PROTAC compounds to bind the polycomb repressive complex 2 (PRC2) or bromodomain-containing protein 4 (BRD4) and target them for degradation in order to reverse latency. The second investigator, Marina Tuyishime (Duke University, Durham, USA), analysed 61 different combinations of antibodies to determine which one resulted in the most efficient NK-cell killing of primary cells infected *in vitro* with HIV isolated from resting memory T cells. She found that combinations of three antibodies were the most potent, with inclusion of the non-neutralising antibody, A32, significantly improving cell killing.

Eric Cohen (Université de Montréal, Montreal, Canada) presented an overview of the Canadian HIV Cure Enterprise (CanCURE), funded by the Canadian Institutes of Health Research (CIHR), which has partnered with MDC researchers to develope assays for quantifying, characterising, and determining the age of the reservoir and to use Nef inhibitors to enhance killing of latently infected cells. The current focus of CanCURE 2.0 is to investigate the direct and indirect contribution of myeloid cells to HIV persistence and to develop therapeutic strategies to achieve durable remission.

Basic science of HIV latency

Research on basic mechanisms of viral persistence remained a major focus of the conference. The prevailing view was that HIV-1 enters a latent state in memory CD4+ T cells and, while in a latent state, is not susceptible to immune clearance mechanisms. As such, the latently infected cell acquires the intrinsic longevity of the memory CD4+ T cell and allows lifelong persistence of the virus within the infected host. Investigators have turned their attention to identifying strategies to eliminate these reservoirs. Most of the effort has been to identify small molecules that can reverse viral latency thereby exposing the infected cell to host immune clearance mechanisms as well as boosting antiviral immunity against the infected reservoir cell. Significant progress has been made in orchestrating conditions that recreate viral latency in primary CD4+ T cells *in vitro* and, in addition, investigators

are now looking for signals in latently infected cells that would distinguish them from uninfected ones. Although numerous lines of evidence indicate that the provirus is transcriptionally silent in a latently infected cell (and therefore, unable to directly influence cellular gene expression), it is possible that some basal level of viral transcription might alter the expression profile of the host cell. This line of research is essential if we are to develop strategies that specifically target latently infected cells for removal.

Hannah Sperber (Vitalant Research Institute, San Francisco, USA) described efforts to profile latently infected cells using NanoString and mass cytometry [3]. The investigators used a dual reporter virus that allows latently infected (transcriptionally silent) cells to be purified. They interrogated the latently infected cells using NanoString technology in which an HIV probe is coupled to RNA and protein probes to allow simultaneous detection and quantification of viral RNA and protein. Target primary CD4+ T cell targets were activated and infected with the reporter. After isolation of latently infected cells, NanoString and cytometry time of flight (CyTOF) was used to identify differential expression of cellular genes in latently infected versus productively infected and uninfected cells. Three differential signatures were identified (IL8 mRNA, ENTPD1 mRNA and NT5E protein). The authors hypothesise that this differential expression may be a consequence of hypoxic conditions and adenosine signalling that may be involved in HIV persistence. It remains to be determined whether the physiologic state of latently infected CD4+ T cells created *in vitro* accurately reflects the *in vivo* latent state memory CD4+ T cells.

Eli Boritz (NIH, Bethesda, USA) has been optimising PCR-activated cell sorting (PACS) to enable transcriptomic analysis of latently infected cells *ex vivo* [4]. Since this approach can utilise infected cells, and presumably latently infected cells from people living with HIV-1, it is likely to reflect the physiological state of latent infection. However, the rarity of latently infected cells poses a challenge for this approach. In addition, there are no markers with which to isolate latently infected cells from a background of uninfected cells. The authors have been developing approaches that allow PCR in single cells that are encapsulated in droplets and then sorted on the basis of their HIV signal. This is then followed by RNAseq to identify any differentially expressed genes between latent and productively infected cells. This approach is now being applied to cells from infected individuals and could hopefully provide detailed insight into the transcriptome of the latently infected cell as it exists *in vivo* and perhaps reveal markers that could be specific to latently infected cells.

Nadia Roan (Gladstone Institute of Virology and Immunology, San Francisco, USA) has been taking a different approach to profiling latent infected cells [5]. The approach involves first activating latently infected cells *ex vivo*. This might seem counterintuitive since those cells would be expected to be very different to their original state *in vivo*. However, Dr Roan proposes that some features of the original latently infected cell can be deduced from the nature of its stimulated counterpart. Following *ex vivo* stimulation, the proteomic profiles of the reactivated cells are determined by CyTOF. The investigators then use this information to derive a proteomic atlas that can be used to infer the characteristics of the original infected cell that was presumable in a latent state. The method, when applied to HIV-1 infected individuals on ART, was able to reveal some proteins that appeared to be upregulated in the latent state including PD-1, TIGIT and Ox40. Curiously, cells activated with latency reversal agents had a different profile to those reactivated with PMA and Ionomycin while latent cells from different donors exhibited common features. It remains to be determined whether the phenotype of latently

infected cells identified through this approach is a consequence of the expression of genes induced in the latent state or whether it reflects the phenotype of CD4+ T cells that are more predisposed to latent HIV-1 infection.

Animal model and *In vitro* studies of HIV persistence

The second oral session of the Workshop focused on animal models of HIV persistence and was chaired by Ann Chahroudi of Emory University, and Afam Okoye of the Oregon National Primate Research Center.

J Victor Garcia Martinez (UNC Chapel Hill, USA) first reviewed the contributions that the two primary animal models of HIV infection –NHP and humanised mice – can make to the study of persistent HIV infection [6]. He outlined examples of where both model systems have been used to study four central approaches to attacking persistent, latent HIV infection: (1) stem cell transplantation (as in the Berlin and London patients, cured of HIV infection); (2) modulation of the anti-HIV immune response through the use of a vaccine or immunotherapy (3) gene therapy, or (4) induction of latent HIV and destruction of the infected cell.

Of course, SIV infection of macaques results in persistent infection with progression to AIDS. Three specific species have been the focus of most experimentation in this area: rhesus, pigtailed and cynomolgus macaques, and a limited number of SIV strains have been used for experimentation including SIVmac251, SIVmac239, SIVsmn, and SIV/HIV hybrid termed RT-SHIV. The most widely used and effective ART combination for treatment now consists of injections of dolutegravir, tenofovir, and emtricitabine (FTC).

Professor Garcia Martinez reviewed examples of each of the four cure approaches in the NHP model.

Peterson *et al.* demonstrated that transplantation and CCR5 gene editing is equally feasible in infected and uninfected animals, that edited cells persist, traffic to, and engraft in tissue reservoirs, and that this approach significantly reduces secondary lymphoid tissue viral reservoir size [7]. The Whitney laboratory demonstrated an effect of orally administered Toll-like receptor 7 (TLR7) agonists GS-986 and GS-9620 to reverse SIV latency and induce transient viremia in rhesus macaques infected with SIV and treated with suppressive ART [8]. However, the Lifson laboratory did not find evidence to support a role for robust GS-9620-mediated induction of virus expression and did not observe immune response boosting during the first 12-dose course of GS-9620. In their study, prolonged treatment with cART initiated at time points very early after infection may therefore have the paradoxical effect of limiting the size of the established viral reservoir while also limiting the potential effectiveness of therapies such as TLR7 agonists [9]. The Barouch laboratory reported the possibility that administration of the V3 glycan-dependent bNAb PGT121 together with the TLR7) agonist vesatolimod (GS-9620) during ART initiated in early acute infection might potently target the viral reservoir [10].

Professor Garcia then reviewed parallel experiments in humanised mice. As mice are naturally refractory to HIV infection, in this model immunodeficient mice that will not reject a human cell transplant are reconstituted with human cells and(or) tissues that are susceptible to HIV infection. In these models the targets are the same cells that are infected with HIV in humans. HIV infection of humanised mice results in the establishment of latency and persistence. A variety of models have been developed, those transplanted with CD34 stem cells only, or with thymic/liver

tissue organoids, or 'BLTs' with both stem cells and organoids, and variants of the BLT model engrafted with either only T cells ('TOMs') or myeloid cells ('MOMs'). He showed examples of genetic delivery of the bNAb PGT121 in this model [11], CRISPR editing to destroy HIV genomes in the model [12], and latency reversal via non-canonical NFkB signaling [13]. Most striking, this last study [13], and an additional one using an immunomodulatory approach, a combination of CD8 cell depletion and IL15 signalling [14], found that results in the humanised mouse model were completely concordant with those in parallel NHP studies. Finally, Professor Garcia showed data from new models, in which lung epithelial cells were also included in the humanised mouse transplant, expanding the viral tropism of the model to allow the study of CMV and other human viral pathogens.

Brandon Keele of the AIDS and Cancer Virus Program of the NCI's Frederick laboratory discussed his laboratory's work developing clonal stocks of SIV that include a genetic barcode used to discriminate viral lineages during NHP infection and persistence [15]. These synthetic swarms of virus are consistent between animals, greatly facilitating replication of studies, and identical outside of the genetic barcode. Characterisation of a barcoded SIVmac239 has been reported [16,17], and Dr Keele finds that viral dose at infection is proportional to number of barcodes detected.

Barcoded viruses are functional and the vast majority of barcodes are found replicating *in vivo*. While the number of barcodes found to replicate is dose dependent, viral growth rates are constant regardless of dose. Therefore, both dose and cART timing can be used to alter reservoir size. Barcoded swarms have been made available for several SIVmac239 strains, and the SHIV subtype B and subtype C. Now these are available for studies of reactivation and rebound after ART interruption. Possible new uses in cure/reservoir research for barcoded viruses include studies of initial reservoir establishment, maintenance of persistent infection, the source of intermittent viremia, studies of clonal expansion, rebound rates, reseeding and population shifts over time. Most significantly, studies thus far suggest that time to detectable viremia (rebound after ATI) measures only the first successful reactivation event, and therefore has limited dynamic range in its ability to reflect changes in reservoir size. Therefore interventions that have a very large impact on reservoir size might lead to longer time to rebound, but more direct measures may be preferable, although difficult.

Tania Immonen of the same laboratory group then reported the use of such barcoded virus (SIVmac239M) to track changes to the composition of the rebound-competent reservoir, and assessed the reactivation of individual viral lineages during successive analytic treatment interruptions (ATIs) [18]. Four rhesus macaques were infected with SIVmac239M, and ART initiated at 10 days post infection. Three ATIs of 21 days or less were initiated at 313, 444 and 682 dpi in each animal, with the second and third ATI preceded by antibody-mediated, CD8+ lymphocyte depletion 3 days prior to cessation of treatment. Plasma viremia was suppressed to below 15 copies/mL for at least 3 months between ATIs, and ART was resumed after peak viremia was reached during viral recrudescence. Next generation sequencing was used to assess the distribution of barcodes in plasma during primary infection and during each viral rebound.

Infection with many distinct barcodes was established in each animal, with a mean of 599 (557 to 650) barcodes observed pre-ART (10 dpi). The magnitude and diversity of viral recrudescence was the highest during the second ATI in each animal, with a mean of 109 (89 to 133) detectable lineages *vs* 22 (8 to 34) and 27 (8 to 37) barcodes during the first and third ATIs,

respectively. The probability of individual barcode detection during each ATI increased proportionally with their frequencies at 10 dpi, demonstrating the establishment of a large replication-competent reservoir representative of pre-ART viral lineages. However, in three out of four animals, the proportion of barcodes shared between consecutive ATIs was significantly higher than expected based on their relative frequency before cART, indicating enrichment of reactivated barcodes in the rebound competent reservoir (RCR) following ATI.

Overall, the probability of reactivation of a viral lineage during an ATI is proportional to its frequency in plasma before cART initiation. ATIs can alter the representation of population within the rebound-competent reservoir. This new technique provides an important tool in the NHP model to examine viral dynamics at the individual lineage level.

Denise Hsu presented a study that revisited the approach of Borducci *et al* [10]. This group, led by the Arms Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, administered oral GS-986 TLR-7 agonist every 2 weeks from week 14 to week 32 and intravenous broadly neutralising HIV antibodies N6-LS and PGT121 every 2 weeks from week 24 to 32 (although antibody therapy was limited in some animals by the appearance of 'anti-drug' antibodies against N6-LS or PGT121) in 16 NHP treated with ART 14 days after SHIV-1157ipd3N4 infection [19]. The prior Borducci study had started ART 7 days post SHIV-SF162P3 infection, given TLR-7 agonist and PGT121 antibody, and found delayed viral rebound and induced SHIV remission in some animals.

In this new study, after ART initiation on day 14, SHIV RNA became undetectable in all animals by week 8 and remained undetectable until ART interruption. Animals received 7–10 doses of GS-986 TLR agonist, 2–5 doses of PGT121 antibody and 2–5 doses of N6-LS antibody. Median time to viral rebound was 6 weeks in the active arm and 3 weeks in the control arm (P=0.024). There was no significant difference in post rebound peak or setpoint viremia between groups. GS-986 administration was associated with upregulation of cytokines consistent with human data but no detectable plasma viral blips 24 hours after dosing. The combinatorial use of N6-LS and PGT121 was associated with faster development of anti-antibody response than PGT121 alone. The GS-986/PGT121/N6-LS strategy was associated with greater reduction in SHIV DNA, and a modest delay in rebound.

Chris Peterson (Fred Hutchinson Cancer Research Center, Seattle, USA) reported the effect of chimeric antigen receptor T cells and stem cells in SHIV in NHP [20]. Adoptively transferred CAR T cells have enabled long-term remission in cancer and are hoped to confer a durable anti-HIV immune response. The group has previously described a hematopoietic stem and progenitor cell (HSPC)-based CAR approach that supports lifelong persistence of HSPC-derived CAR T cells but is limited by the cytotoxic conditioning regimen that is required for HSPC engraftment. Second generation CD4-based CAR molecules (CD4CAR) were independently developed for primary NHP HSPCs and T cells, and combined with CCR5 editing or virus fusion inhibitors to protect against infection with SHIV. Where applicable, SHIV-infected animals were suppressed by ART for at least 1 year prior to cell therapy. CD4CAR T-cell products were infused without a conditioning regimen. CD4CAR HSPC studies were designed to identify the reduced-intensity conditioning regimen with the highest ratio of antiviral impact to cytotoxicity.

Infusion of cell-based antigen significantly increased the percentage of peripheral blood CAR+ T cells, which persisted *in vivo* and significantly delayed SHIV rebound following ART withdrawal.

Plasma viremia in one animal remained at the limit of detection 60 days post-ART treatment interruption (ATI). CD4CAR HSPC products engrafted following busulfan conditioning with minimal toxicity, and busulfan/CD4CAR-HSPC animals resisted repeated low-dose intrarectal SHIV challenges.

These studies demonstrate expansion of virus-specific, autologous CAR T cells in infected, suppressed hosts, and show promise in conferring antiviral immune response following CAR T cell therapy. These preclinical findings are already under investigation in analogous clinical trials utilising CAR T cells (University of Pennsylvania NCT03617198) and busulfan/gene-edited HSPCs (City of Hope NCT02500849).

Maud Mavigner (Emory and Yerkes Primate Research Center, USA) presented recent findings originating from the Silvestri group [21]. Previous studies suggested that antibody-mediated, therapeutic, transient depletion of CD8 T cells might induce latency reversal in CD4 T cells, by unknown mechanisms. In this study, the effect of CD8 depletion was tested in combination with a 'typical' latency reversal agent, AZD5582. This drug is a SMAC mimetic or inhibitor of apoptosis protein (IAP) inhibitor. These small molecules induce signalling via the non-canonical NF-kB pathway (ncNF-kB), a promising LRA approach [13]. Furthermore, as CD8+ cells contribute to the maintenance of viral suppression during ART, experimental depletion of CD8+ cells may act synergistically with LRAs to induce virus production.

Eighteen SIVmac239-infected macaques on ART for 55–67 weeks were administered weekly intravenous doses of AZD5582 at 100 mg/kg. Six animals also received a single dose of the CD8α-depleting antibody MT-807R1 at 50 mg/kg 24 hours prior to AZD5582 treatment. On-ART plasma viral loads were monitored to assess for latency reversal and single genome sequence analysis of the *env* gene of SIVmac239 was performed in the plasma.

On-ART viremia of >60 copies/mL was observed in 5/12 (42%) rhesus macaques (RMs) treated with AZD5582 only. Ultrasensitive plasma viral load assessment (>3 copies SIV RNA per mL of plasma) showed increased plasma viremia in three additional RMs treated with AZD5582. When experimental depletion of CD8a+ cells was combined with AZD5582 treatment, 6/6 (100%) RMs demonstrated on-ART viremia of >60 copies/mL. These episodes of viremia >60 copies/mL were seen in 25% *vs* 16.7% of viral load measurements for the CD8-depleted+AZD5582 group *vs* the AZD5582 only group, respectively. Further studies are needed to understand how CD8 depletion contributes to latency reversal, so that the approach may contribute to such efforts in human studies.

Finally, Tom Hope (Northwestern, Chicago, USA) presented visually stunning studies using PET/CT and multiscale imaging to study real-time dynamics of SIV-infected cells in primates, following infection, early ART initiation and rebound after analytic treatment interruption [22]. Viruses could be visualised as they were labelled with SIV envelope Ab carrying the PET/CT $_{64}$Cu-FAB2 probe (7D3) which allows iterative imaging of the same animal to quantitatively localise SIV after initiation and cessation of ART.

Rhesus macaques were challenged both vaginally and rectally with a single high-dose of SIVmac239ff (containing a firefly luciferase reporter) and suppressive ART was initiated 4 days post-challenge. Following at least 6 months of ART, treatment was discontinued and RMs were necropsied at days 4, 5, 7 and 10 post-ART cessation. During and after ART cessation, PET/CT scans revealed the sites of SIV gene expression. This was validated by using the PET/CT signal to guide the isolation of small pieces

of tissues at the time of necropsy that contained SIVmac239 infected cells by PCR and immunofluorescence staining.

SIV infections sites were efficiently detected by PET/CT as early as 4 days post-ART cessation in multiple tissues particularly in the genital tract, small bowel and colon, spleen, lymph nodes, and unexpectedly the heart. Interestingly, rebound was primarily observed in the same anatomical sites where signal was localised after initiation of ART. Initial phenotyping studies of rebound site have surprisingly only revealed SIV Env and Gag positive myeloid cells. No infected CD4+ T cells have been detected although they are abundant in the area of rebound often in contact with the SIV expressing myeloid cells. This restriction of the early rebound to tissue resident myeloid cells may represent a bottle neck, and potential vulnerability, required for virus replication independent of the source of the reservoir. Alternatively, it may be that the density of antigen is sufficient in myeloid cells to allow detection by this method, and envelope in or on T cells is too rare to be seen. Nevertheless, correlative PET/CT is a powerful new tool to define the anatomy and physiology of HIV replication and re-emergence.

Virology of HIV persistence

The session on virology of HIV persistence began with a presentation by Pamela Skinner (Professor of Veterinary and Biomedical Sciences, University of Minnesota. St. Paul, USA) who explored the possibility of targeting the germinal centres (GC) of rRM lymph nodes in a model of SIV infection [23]. GC are indeed credited with among the highest concentrations of virions associated with follicular dendritic cells (FDC) together with infected CD4+ T follicular helper (fh) lymphocytes. The investigators hypothesised that the artificial expression of CXCR5 (the chemokine receptor required to be expressed at the cell surface in order to enter the GC) by transduction of virus-specific T cells would enhance their capacity to access the lymph node follicles. They used a vector designed by Ed Berger at NIAID (CD4-MBL-CAR/CXCR5) conferring the capacity, on the one hand, of homing to B cell follicles and, on the other hand, of recognising and killing infected cells by expression of a CAR. The construct was delivered to peripheral blood cells by gamma retroviral vectors and the cells expressing the CAR were indeed observed to proliferate in the lymph node and make contact with cells expressing HIV RNA in the B cell follicles. In addition to this morphological evidence, transduced cells controlled SIV-infected cells better than mock-transduced cells *in vitro*.

Michael J. Peluso (Division of HIV, Infectious Diseases, and Global Medicine, University of California San Francisco, USA), member of the SCOPE IPDA Study team, compared the decay rate of intact versus defective proviral sequences in infected individuals on suppressive cART [24]. The study was based on the intact proviral DNA assay (IPDA) allowing the simultaneous analysis of proviral packaging signal and *env* regions based on the assumption that replication-competent proviruses can be amplified at both regions whereas defective proviruses either amplify at a single region or do not amplify at all [25]. This study aimed to investigate the dynamics of the proviral reservoir in a longitudinal cohort of 81 individuals on suppressive cART for at least 12 months, with negative plasma viremia levels maintained for at least 2 following years. The investigators observed that intact proviral sequences displayed a faster decay rate in comparison to the defective proviruses, as already anticipated by earlier QVOA studies. Dr Peluso also reported that proviral load in peripheral blood cells correlated with a higher nadir of CD4+ T cell counts and with the CD4/CD8 ratio whereas, of interest, age, race,

gender, duration of infection, protective HLA alleles or CCR5 heterozygosity had no substantial effect.

Jonathan Karn (CWRU/UH Center for AIDS Research, Case Western Reserve University, Cleveland, USA) discussed the role of sexual hormones in the HIV reservoir of women living with HIV, based on his earlier findings indicating that oestrogens inhibit proviral transcription and virus replication in T cell lines, and that women tend to have a smaller reservoir of infected cells in comparison to age-matched men [26,27]. Furthermore, Karn emphasised that in women's reproductive window the HIV reservoir increases in the secretory phase of the menstrual cycle, when the levels of oestrogen decrease and progesterone dominates. By studying the Chicago WIHS cohort and the ACTG NWCS 443 cohort of UCSD, Dr Karn observed that women's ageing is associated with an increase HIV reservoir. Furthermore, high levels of cell-associated HIV RNA (both basal and after Concanavalin A stimulation) were typically observed in cells of women in their post-menopausal phase (ACTG 5366 cohort) in correlation with a pattern of increased histone acetylation.

Francesco Simonetti (Johns Hopkins School of Medicine, Baltimore, USA) investigated the potential role of antigenic stimulation in the expansion of HIV-infected cell clones specific for cytomegalovirus antigens on the assumption that chronic viral infections represent good models to study antigen-driven expansion of cell clones carrying integrated proviruses [28]. He indeed provided evidence of antigen-driven clonal selection of HIV-infected cells, including both *bona fide* replication-competent and defective viral sequences, some of which integrated with 'hot spots' in host cell DNA previously identified by different groups including BACH-2 and STAT5b.

Mathieu Dubé (University of Montreal and CR-CHUM, Canada) discussed whether by applying dual gagRNA and p24 protein detection by RNAflow-FISH it would be possible to identify cells infected with translational-competent proviruses [29]. He reported that p24 Gag+ cells (enriched in the effector memory subset) represent a minor fraction of the inducible reservoir upon *ex vivo* cell stimulation with PMA+ionomycin, whereas most infected cells are characterised by incomplete viral transcription.

Cristina Gàlvez (IrsiCaixa AIDS Research Institute, Badalona, Spain) described the existence of a rare population of infected individuals characterised by a very low HIV reservoir within individuals receiving cART very early after infection, a group of 12 individuals defined as 'low viral reservoir treated' (LoViReT) within a cohort of 453 individuals on cART for ≥3 years [30]. The investigators compared them to 13 control 'standard progressor' individuals. LoViReT showed almost 1 $\log_{10}$ lower levels of peripheral blood associated total HIV DNA in spite of similar levels of plasma viremia, measured before initiation of cART, of control individuals. Lower levels of HIV DNA were also noted in LoViReT 18 months after the initiation of cART together with a faster decay of HIV DNA, observed after 5 years of cART. When cells were isolated by leukapheresis, replication-competent virus were not rescued in about 70% of LoViReT, whereas both lymph node and rectal biopsies did not show evidence of HIV DNA. When peripheral blood-derived CD4+ T lymphocytes were isolated from LoViReT, a partially different pattern of infection was observed in that most HIV DNA+ cells were found in transitional memory (TM) and effector memory (EM) cells in analogy to that observed in ART-naïve HIV controllers and in post-treatment controllers. Gàlvez proposed that LoViReT are the consequence of a natural predisposition to better control HIV infection in the absence of therapy combined with a boosting of this predisposition induced by cART.

Immunology of HIV persistence

While it is known that a fraction of HIV-infected individuals named 'elite controllers' can naturally control HIV replication, differences in the T-cell epitopes recognised between controllers and non-controllers have not been fully characterised yet. Using viral sequences obtained from people living with HIV and by applying a method called viral fitness landscapes, Bruce Walker (Ragon Institute, Cambridge, USA) showed that epitopes commonly targeted by T cell responses in elite controllers contain residues that are multidimensionally conserved [31]. They created an algorithm to quantitate the 'network score' of HIV epitopes, which reflects the importance of these small peptides in the maintenance of the structure of given HIV proteins. Strikingly, they observed that elite controllers target more network epitopes compared to progressors. CTL epitope network scores differentiated controllers from progressors, independent of host HLA. These observations suggest that the development of a vaccine using highly networked epitopes as immunogens may convert progressors into controllers.

Using samples from the acute infection study RV254/SEARCH011 in Bangkok, Nicolas Chomont (Université de Montréal, Montreal, Canada) demonstrated that a large pool of HIV infected cells is established during the first few weeks of infection [32]. T-cell receptor (TCR) sequences of individual p24+ cells revealed that clonal expansions were rare in the pool of infected cells at this early stage: All infected cells (both in blood and lymph nodes) expressed different TCRs, indicating independent events of infection. Upon ART initiation during acute infection, the majority of these cells were cleared, whereas the pool of infected cells was remarkably stable in individuals starting ART during chronic infection. During ART, clonal expansions in the reservoir were frequent. Expanded reservoir cells were enriched in transitional and effector memory cells but also present at low frequencies in central memory cells, suggesting that this long lived subset with remarkable self-renewal capacities could be the source of proliferating infected cells during ART.

Michael Peluso (University of California San Francisco, USA) identified a subset of elite controllers with a very small viral reservoir and who were less likely to progress [33]. These 'exceptional elite controllers' were defined as having HIV DNA levels below the limit of detection of the HIV DNA quantification assay and had very low levels of cell associated HIV RNA. These individuals were more likely to carry HLA protective alleles than the general population of elite controllers and had lower levels of antibodies to several HIV proteins including matrix, integrase and protease. However, frequencies of activated CD8+ T cells and PD-1+ memory CD8+ T cells did not differ between the two groups. Finally, exceptional elite controllers had a marked reduced risk of losing virological control compared to elite controllers. Dr Peluso concluded that these rare individuals may be less likely to progress and may not need ART.

By Taking advantage of the uniqueness of the TCR sequences in distinct T cell clones, Pierre Gantner (Université de Montréal, Montreal, Canada) demonstrated that clonal expansion within the pool of infected cells (defined as p24-expressing cells after stimulation) was common in individuals on ART [34]. Using longitudinal samples from eight individuals on ART, he observed that some of these expanded clonotypes had the ability to expand and contract over time, indicating that the reservoir is dynamic even during suppressive ART. Expanded T cell clonotypes systematically displayed several memory phenotypes, including a central memory phenotype, albeit at low frequencies compared to transitional and effector memory cells. This suggested that these cells were infected at an early stage of differentiation and subsequently differentiated during cell proliferation, likely in response to antigen stimulation.

Using a similar approach (cell sorting of individual p24+ cells from virally suppressed participants on ART), Caroline Dufour (Université de Montréal, Montreal, Canada) analysed the integrity of the expanded viral clones within the reservoir [35]. Near full-length HIV genomes were sequenced using a modified version of the FLIPS assay and the phenotype of these individual cells were analysed and associated with specific defects in the viral genome. The majority of the sequences obtained were found in multiples copies, suggesting clonal expansions in the reservoir. There was a large diversity in the phenotypes of cells harbouring the same proviral sequence. The few cells (n=4) harbouring intact proviral sequences did not show a distinct phenotype compared to the cells harbouring defective proviruses. Altogether these results indicate that expanded infected cells can display diverse phenotypes and suggest that cells harbouring intact genomes are indistinguishable from the defective reservoir cells.

Asier Sáez-Cirión (Institut Pasteur, Paris, France) presented unique results from the IciStem study regarding T cell responses in HIV infected individuals who had received allogeneic stem cell transplant (allo-HSCT) [36]. He emphasised that allo-HSCT is the only medical intervention that consistently and substantially reduces the HIV reservoir and has led to a potential cure for HIV infection. Accordingly, allo-HSCT considerably reduced HIV DNA levels in the participants of the IciStem study. There were high levels of T-cell activation observed a few weeks post allo-HSCT, which may allow reseeding of the viral reservoir. HIV-specific CD8+ T cell responses were primed after HSCT, indicating that HIV antigens were produced during T cell expansion. These responses persisted over time but were not polyfunctional, suggesting that they would probably be insufficient to control viral replication if ART was interrupted. Whereas HIV-negative controls receiving allo-HSCT typically normalised their CD4/CD8 ratio after 1–2 years, the ratio remained <1 in IciStem participants up to 7 years after the intervention.

Eva M Stevenson (Weill Cornell Medicine, New York, USA) characterised HIV-specific T cell responses in individuals on ART for >4 years [37]. Using a large number of samples from the ACTG A5321 study, she found an association between the levels of HIV DNA and Nef/Tat/Rev-specific T cell responses. Interestingly, there were no significant associations between the size of the reservoir and T cell responses to other gene products. In a longitudinal analysis, HIV-specific T cell responses had long, median half-lives that differed by recognised gene product. Higher levels of HIV DNA at study entry (median 7 years on ART) were associated with lesser levels of decay of Nef/Tat/Rev specific T cell responses, suggesting ongoing antigen stimulation of these cells over years. Detection of granzyme B expressing cells indicated that some of these cells had HIV-specific cytotoxic function during long-term ART. Unexpectedly, HIV-specific T cell response magnitudes differed between male and female participants, with female displaying 1.8 to 4.7-fold greater T cell responses. Whether these persistent HIV-specific responses have the ability to clear reservoir cells *in vivo* remains to be determined.

Human studies and drug development

The fifth and seventh oral session of the Workshop focused on human studies and drug development and were respectively chaired by Bonnie Howell of Infectious Disease and Vaccines Merck, Pennsylvania/Javier Martinez Picado of IrsiCaixa, Barcelona and Christina Psomas from the European Hospital Marseille,

France/David Smith of University of California, San Diego School of Medicine, La Jolla.

Richard Dunham (ViiV Healthcare and UNC-CH Région de Raleigh-Durham, NC-USA) described novel latency reversing agents, namely inhibitors of apoptosis protein inhibitors (IAPi) that are more focused than previous 'induce and reduce strategies for HIV cure' such as protein kinase C agonists (PKCa) [38]. Indeed, target activation of T cells by PKCa such as ingenol B and GSK molecule GSK 445A has been validated *in vitro*, as well as *in vivo* in the RM model and induces broad cellular activation through the canonical NF-kB pathway, but efficacy and systemic toxicity are observed at nearly the same doses. IAPi such as molecule AZD5582, which is a second mitochondria-derived activator of caspases (SMAC) mimetic), also induce replication competent HIV in diverse model systems (cell line models of HIV latency, primary CD4+ T cells, as well as in BLT humanised mouse models and RMs). IAPi activate NF-kB through the non-canonical pathway that induces more selectively HIV expression (plasma viremia and tissue viral RNA) with minimal off target effects thanks to a more limited transcriptional response [39].

Lars Pache (Sanford Burnham Preis Medical Discovery Institute, La Jolla, USA) outlined findings on optimisation of SMAC mimetics, some pro-apoptotic mitochondrial proteins of the IAPi family that were initially used for cancer therapy, as HIV-1 latency reversing agents [40]. Second generation SMAC mimetics such as compound SBI-0953294 show dramatically improved LRA efficacy and potency. SBI-0953294 does not induce rCD4+ T Cell activation or cytokine release in PBMC or rCD4+ T cells. Rodent study shows favourable PK and target engagement properties of SBI-0953294 (plasma viremia detected in 3/9 mice 48h after treatment), suitable for *in vivo* application. No adverse effects were observed in mice treated at concentrations sufficient to mediate sustained target engagement. Data presented by Dr Pache provided proof-of-concept for HIV latency reversal by SBI-0953294 in BLT humanised mouse model in the absence of T cell activation.

Joseph Hesselgesser (Gilead, Foster City, CA-USA) presented an early phase 1b dose-escalation study of vesatolimob (VES) administration in people living with HIV (PLWH) on ART in the context of Gilead's HIV remission programme where combinations of VES, effector-enhanced anti-envelope antibodies and CD8 based vaccines are considered [41]. Pharmacodynamics responses, plasma viremia and reservoir measurements (total cell associated viral DNA or CAVD, intact proviral DNA assay or IPDA and inducible virus production assay) were presented, establishing safe and immune active VES doses ≥6mg.

Jonathan Li (Harvard Medical School, Boston, USA) analysed viral composition during periods of low-level viremia in 20 HIV post-treatment controllers (PTCs) and 13 post-treatment non-controllers (NCs) from AIDS Clinical Trials Group (ACTG) ATI trials [42]. Using single-genome sequences (SGSs) amplification of HIV-1 pol after HIV RNA extraction the group analysed, in >1200 plasma sequences, evidence of HIV sequence evolution in terms of increased sequence divergence, increased root-to-tip distance (nucleotide substitution per site), change in population structure (panmixia) and increased proportion of HLA escape. PTCs exhibited sustained HIV remission despite evidence of plasma divergence and evolution. Over time, increasing viral diversity was detected in almost all PTCs, but rates of diversification were significantly slower in PTCs compared to NCs (median 0.05% *vs* 0.27% per year, *P*=0.007). There was no evidence of enrichment of protective HLA alleles in PTCs, but the increasing frequency of HLA-escape mutations in a subset of PTCs could indicate the presence of some T cell-mediated immune pressure. A proportion of HLA-escape mutations were common in HIV sequences from PTCs and not significantly different than NCs (47% *vs* 59%, *P*=0.16).

The detection of dual HIV infection (populations of HIV variants with ≥5% sequence divergence) in a subset of PTCs suggests the presence of an antiviral response that can control a diverse viral population.

Jeffrey Johnson (CDC, Atlanta, USA) assessed virus dynamics and genetic variance of HIV particles in semen relative to peripheral blood after initiating INSTI-based regimens [43]. Semen, blood and rectal swabs for virological examination (virion immunocapture steps followed by Sanger sequencing) were provided by men living with HIV on highly suppressive INSTI-based regimens for >6 months (*n*=15, group A) or ≤6 months (*n*=5, group B) recruited at Fenway Health. Immunocapture assays provide the advantage of both concentrating low-expressing virions and segregating variants from other compartmental virions and circulating (blood) infiltration, while giving hints of possible cellular sources of variant populations (by targeting host cell-type proteins embedded in the virion envelopes).

Five out of 15 men in group A had blood plasma virus at <40 copies/mL, the remaining had an undetectable viral load (VL). All five men in group B had undetectable peripheral blood VL. Durations of infection were >3–28 years.

Immunocapture of virus particles was still possible in 50% of semen from men with low (<40 copies/mL) or undetectable blood plasma HIV RNA by commercial viral load assays. Most successful virion captures from semen were against myeloid cell-associated markers and negative for the CD3+ marker (T cell-derived). The CD3- seminal viruses contained the cellular markers Iba-1 (macrophage source), CD11c (resident dendritic cell source), HLA-DR (activated cell source) and CD16 (migratory monocyte or tissue-resident macrophage source). The virion sequences obtained from the cellular marker captures were largely clonal and varied in both polymorphisms and drug resistance mutations. Reverse transcriptase drug resistance mutations (DRMs) from prior regimens were more prevalent in the semen and distinct from any mutations detected in lymphoid-derived virions.

For all participants, at least one seminal variant was identified with a genetic distance >1.5% in the RT region relative to blood. The greatest genetic distance between a seminal and blood variant was 9% (29 of 318 nucleotides differed). Seminal virions containing HLA-DR and CD16 appeared to be more closely genetically linked to the blood viruses that were detectable.

Even if suppressive therapy significantly prevents HIV sexual transmission, on the basis of 'Undetectable = Untransmittable (U=U)', the large genetic distances in semen relative to blood, however, have implications for assessing genotypic linkage if insufficient suppression leads to their transmission.

Thomas Rasmussen (Peter Doherty Institute for Infection and Immunity, University of Melbourne and Royal Melbourne Hospital, Australia) interestingly presented results of the AMC-095 study regarding the impact of single versus combined immune checkpoint blockade (ICB) of PD-1 and CTLA-4 as cancer immunotherapy on the HIV reservoir *in vivo* [44]. The AMC-095 study is an ongoing prospective multisite (31 sites in the US and Australia) non-randomised phase I clinical trial of Nivolumab (anti-PD-1) and Ipilimumab (anti-CTLA-4) in advanced HIV-associated solid tumours and classical Hodgkin lymphoma. It includes an HIV reservoir substudy knowing that ICB reinvigorate exhausted T cells enhancing HIV-specific T cell function and may also reverse HIV latency.

Forty participants were included (36 males). Of those, 33 received anti-PD-1 alone and 7 received anti-PD-1 plus anti-CTLA-4. At baseline, median age was 53.0 (Interquartile range [IQR] 47.0–58.5) years and CD4 count was 315 (IQR 227–465) cells/mm^3. Whereas CA US HIV-RNA did not change from baseline in those receiving anti-PD-1 alone, they detected a median 1.44 (IQR 1.16–1.89) fold-increase within 24 hours of the first dose in participants on combination ICB (P=0.031). This increase was also significantly higher compared to the corresponding change from baseline in those on anti-PD-1 alone (P=0.025). Globally they detected no changes in the level of HIV DNA (transient 30% decrease at day 1 and 7 but not sustained beyond cycle 1, P=0.01 and P=0.03 respectively) or the frequency of cells containing replication-competent HIV, but in both of two individuals on combination ICB with samples available for QVOA, the frequency of replication-competent HIV decreased. Dual ICB with anti-PD-1 and anti-CTLA-4 suggested a larger increase in CA-US HIV RNA than anti-PD-1 alone and may potentially impact the frequency of cells containing replication-competent HIV. Improved formulations or a better understanding of toxicities may be of great interest in the future in this indication.

Joshua Cyktor (Massachusetts General Hospital, Boston, USA) described results of the intact proviral DNA assay (IPDA) as a new, more specific ddPCR-based measure of the replication-competent HIV reservoir correlating this technique with other measures of HIV persistence or with immune activation [45]. Participants in ACTG A5321 with chronic HIV and virologic suppression on ART had the following measurements performed on blood samples: intact proviral DNA (IPD) [25], total proviral DNA (sum of defective, hypermutated, intact proviruses), total HIV DNA by qPCR [46], cell-associated HIV RNA (CA-RNA), plasma HIV RNA single copy assay (SCA), T cell activation, and inflammation (including IL-6, IP-10, sCD14, sCD163). Longitudinal measurements of HIV persistence in well-suppressed individuals on ART is crucial, especially in order to evaluate efficacy of different cure strategies. Fifty participants (26% female) of the ACTG A5321 HIV reservoir cohort (AHRC) were evaluated. Intact proviral DNA levels declined significantly between time point 1 at A5321 entry (median 7 years after ART initiation)(n=50) and time point 2 (approximately 4 years after timepoint 1)(n=48): median 57 and 41 copies/million CD4-cells, respectively; P<0.001. By contrast, total proviral DNA was stable: median 551 and 580 copies/million CD4-cells, respectively. Estimated half-life of decline for intact proviral DNA (n=44 participants) was 6.5 years (95% CI 4.5–11.2), whereas half-life for total proviral DNA was 22.9 years (95% CI 11.1–60.9). A subset of individuals had a decline in intact proviral DNA to undetectable levels. IPD levels correlate with HIV pol DNA, CA HIV pol RNA, plasma RNA by SCA and Th2 cytokines (IL-4 and IL-13), but not with inflammatory biomarkers or T cell activation/exhaustion. The overall decline in intact proviruses suggests that cells containing rebound-competent proviruses are diminishing with time. The more dynamic nature of the intact proviral landscape, compared with total proviral HIV DNA, supports the use of the IPDA to assess the impact of interventions targeting the HIV reservoir.

Timothy Henrich (University of California San Francisco, USA) described modalities of use of whole body imaging in HIV persistence and its clinical applications [47]. PET imaging techniques have the potential to characterise tissue HIV virus and infection-related morbidity, monitoring for instance total-body AIDS-virus burden (FDG-PET) [48], longitudinal and spatial dynamics of T cell activation/immune responses ([^{18}F]F-AraG – activated t-cell imaging) during different stages of infection. It reveals difference in whole-body T cell activation/proliferation between uninfected

and ART-suppressed individuals. ImmunoPET with radiolabelled VRC01 bNAbs reveals increased uptake in various tissues/anatomical regions in viremic and ART suppressed participants compared with uninfected controls.

Determining T cell response and imaging viral rebound may be of great interest during ATI [49,50], LRA therapy, immune checkpoint blockade or other immunomodulator therapies, as well as vaccine responses.

Leila Giron (Wistar Institute, Philadelphia, USA) presented results in the field of research of pre-analytic treatment interruption (ATI) biomarkers of viral rebound and viral set point in order to mitigate the risk of ATI in the HIV cure field [51]. The Abdel-Mohsen Laboratory previously reported that galactosylated, bulk IgG glycans negatively correlate with cell-associated HIV DNA and RNA during ART [52]. Using capillary electrophoresis and a lectin microarray, they profiled the circulating glycomic signatures (plasma and bulk IgG) of two geographically-distinct cohorts: (1) Philadelphia Cohort – 24 HIV-infected, ART-suppressed individuals who had participated in an open-ended ATI study without concurrent immunomodulatory agents; and (2) Johannesburg Cohort, serving as a validation cohort of 23 HIV-infected, ART-suppressed individuals who had participated in a 2-week ATI. Higher pre-ATI levels of the IgG glycan, G2, were significantly associated with a longer time-to-viral-rebound (hazard ratio [HR]=0.12, P=0.05) in the Philadelphia cohort. G2 glycan levels were significantly lower at viral-rebound (P=0.02) and viral set-point (P=0.009) compared to their pre-ATI levels. In addition to G2, they identified several predictive glycomic traits in plasma, e.g. levels of FA2BG1, a non-sialylated, core-fucosylated glycomic trait, strongly associated with a longer time-to-viral-rebound (HR=0.023, P=0.05), whereas FA2G2S1, a sialylated glycomic trait, strongly associated with a shorter time-to-viral-rebound (HR=24.1, P=0.028). Additionally, pre-ATI plasma glycomic signatures associated with lower viral setpoint, e.g. T-antigen (Galβ1-3GalNAc) (r=0.75, P=0.0007), or higher viral setpoint, e.g. polylactosamine (r=-0.58, P=0.01). These results were validated in the Johannesburg validation cohort.

Antibody di-galactosylation (G2) predicted time to HIV rebound after treatment interruption. Plasma and antibody glycomic biomarkers predict time to HIV rebound and viral setpoint in two geographically distinct cohorts.

Rasmi Thomas (US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, USA) identified new candidates for host cellular proteins that may impact reservoir size *in vivo* or reactivation during ART [53]. The researchers used next-generation sequencing technologies to investigate cell-specific, transcriptome-wide host gene expression variation influencing the size of viral reservoirs. Peripheral blood mononuclear cells were available from 14 virally suppressed individuals with variable levels of HIV viral DNA following 48 weeks of therapy initiated during Fiebig III acute infection. High quality gene expression and paired immune receptor sequence data were captured from approximately 63,000 cells across 14 donors. They detected 22 major cell populations within this dataset, including all major T cells, NK cells, monocytes, naïve/memory B cells, plasma blasts, DCs and megakaryocytes. The monocyte and CD8+ effector/memory T cell populations had the highest number of significantly differentially expressed genes between the high and low reservoir groups, with the top differentially expressed genes in CD14+ monocytes (THBS1 and IL1beta). These molecules have already been studied in the context of HIV-infection, with a higher THBS1 and IL1beta expression for instance in LTNPs [54,55]. Looking for phenotypic populations that interacted with both THBS1 and

IL1B and associated with lower reservoir size revealed that interaction with central memory CD4 T cells predicted reservoir size. More specifically, increased expression of THBS1 or IL1B in monocytes in the presence of higher frequencies of central memory CD4 T cells predicted lower reservoir size (for every unit increase in the interaction between THBS1/IL1B gene expression levels and frequencies of central memory CD4 T cell, a 24.83 or 68.20 unit decrease in reservoir size was predicted).

New therapeutic approaches

A full sterilising cure does not seem to be in our immediate reach, however therapeutic approaches aiming at a functional cure (where the virus will be maintained below a certain threshold and both morbidity or secondary transmission will not occur) are the focus of current approaches. To be able to expect long-lasting virus remission, interventions should mimic elite or post-treatment controllers with an RNA plasma viral load below the level of detection and persisting at least for 1 year in order to be clinically meaningful and economically sound. Furthermore, to assess the clinical value of these cure strategies, ART interruption remains the only read-out to evaluate viral control after ART discontinuation. Therefore, consensus on the mitigation of these risks following ART interruption requested for the participants and their sexual partners has to be optimised [56]. To this end, identification of inflammatory biomarkers that can predict viral rebound has become a research priority to further reduce risk of participating in cure research clinical trials [57,58]. Several innovative functional cure strategies have been presented this year.

Can we transfer the success of adoptive immunotherapy using chimeric antigen receptor (CAR) modified T-cells from refractory leukemia/lymphoma to HIV infection?

Kim Anthony-Gonda *et al.* (Lentigen, a Miltenyi Biotec Company, Gaithersburg, USA) aimed to engineer a second generation of HIV-1-based lentiviral vectors (LV) encoding anti-HIV CARs targeting well-conserved sites on the Env using two-molecule architecture to improve breadth and resistance to HIV infection [59]. Investigators challenged CAR-T cells with donor-matched PBMCs infected with a global panel of replication-competent HIV infectious molecular clones that encode a *Renilla* luciferase reporter (Env-IMC-LucR). HIV infection suppression was quantified using the LucR activity present in HIV-infected PBMC co-cultures or humanised mice relative to unmodified T cells after HIV infection. Encouragingly, in humanised mice the multispecific duoCAR-T cell therapy was broadly reactive, protective for HIV infection and superior to monoCAR-T cell therapy, paving the way towards a phase I clinical trial for a functional cure.

Similary for cancer microenvironments, specific CTL are kept at bay from B cell follicles of germinal centres where much virus replication occurs and persists on ART. It is well established that a high frequency of follicular SIV-specific CTL inversely correlates with the level of follicular viral replication and with plasma viral load. Therefore, there is a strong rationale for targeting the follicular viral reservoir using T cells co-expressing the B cell follicle homing molecule CXCR5 with an SIV-specific chimeric antigen receptor (CD4-MBL-CAR). H Abdelaal and E Berger *et al.* (University of Minnesota, Minneapolis, and NIH, Bethesda, USA) proposed that CAR/CXCR5 T cells can enter into B cell follicles to clear virus-infected cells [60]. To this end autologous T cells were modified to express CAR/CXCR5 T cells and were infused in six ART-treated SIV-infected rhesus macaques on the day of ART interruption. Three untreated SIV-infected animals were studied as controls. RNAScope, *in situ* hybridisation combined with immunohistochemistry on lymphoid tissue sections were carried out to assess the location, abundance, and persistence of the CAR/CXCR5 T cells as well as the location of SIV-infected cells. Encouragingly, CAR/CXCR5 T cells were able to home inside the B cell follicle and interacted with infected cells. Four out of six animals showed lower viremia compared to the control group and highest level of CAR/CXCR5 T cells occurred 2 weeks after ART discontinuation. These findings support the development of CAR/CXCR5 immunotherapy as a functional cure strategy for HIV infection.

Engineered eCD4-Ig is a fusion of CD4-Ig with a coreceptor-mimetic peptide acting as an antibody-like entry inhibitor that closely mimics HIV-1 receptors [61]. M. Gardner, M. Davis-Gardner, M Farzan from Scripps Research Institute, Jupiter, USA have showed that eCD4-Ig is more potent than CD4-Ig, with neutralisation efficiencies reaching those of broadly neutralising antibodies [56]. They have shown that a RM version of eCD4-Ig (rh-eCD4-Ig) can be expressed using adeno-associated virus delivery vectors (AVV) protecting from SHIV-AD8 and SIVmac239 challenge. Now, they assessed the ability of AAV-expressed rh-eCD4-Ig to suppress SHIV in macaques after holding ART [62]. Six macaques were inoculated with AAV8/AAV1 vectors encoding rh-eCD4-Ig and four others were left untreated as controls. ART was stopped 2–4 weeks after the second AAV inoculation. Following 1–4 weeks after ART discontinuation, all macaques had viral rebound, although those treated with AAV-rh-eCD4-Ig had a much lower rebound viremia. After 2 years the six macaques remained with a plasma VL <100 viral RNA copies/mL while control animals ranged 1400–7000 RNA copies/mL. Importantly, the six treated macaques have been expressing high level of rh-eCD4-Ig throughout the 2 year-long study. High level of expressed rh-eCD4-Ig represents a promising strategy for a functional cure.

ABX464 is a first-in-class, clinical-stage, small molecule for oral administration that has shown strong anti-inflammatory effects in inflammatory bowel disease while preventing HIV replication [63]. ABX464 acts as viral inhibitor by promoting HIV-RNA splicing events and interfering with the production Rev and Tat proteins. This product also inhibits viral mRNA export required for Gag, Pol, Env production. This novel antiviral binds to the cap binding complex, interfering with splicing and Rev-mediated export of HIV RNA. Interestingly, ABX464 has been showed to delay viral rebound in a humanised mouse model. S Moron-Lopez *et al.* (University of California San Francisco, USA; IrsiCaixa AIDS Research Institute, Barcelona; ABIVAX, Paris; San Francisco VA Medical Center, USA) investigated the effect of ABX464 on the HIV transcription profile and HIV DNA in CD4+ T cells from ART-suppressed participants enrolled in the ABIVAX-005 clinical trial [64]. Eleven participants on ART received 150mg of ABX464 daily for 4 weeks. Total HIV DNA, intact HIV DNA, and read-through total/initiated, polyadenylated and multiply-spliced Tat-Rev HIV transcripts were quantified using ddPCR. A modest decrease in the total HIV DNA was observed with ABX464. Also, intact HIV DNA increased after ABX464 discontinuation. In addition, ABX464 treatment decreased total initiated HIV RNA per million CD4+ T cells and per provirus (HIV RNA/HIV DNA). Study findings suggest that ABX464 may act as an 'ART intensifier' in patients.

Conclusion

The 2019 HIV persistence workshop was driven by outstanding science and new unpublished data presented by young investigators and a panel of experts that summed up the current advances in the field. Participation in this workshop enhanced thinking, new energy, ideas and collaborations in the domain of HIV persistence and reservoirs.

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