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Highlights from the Fourth Biennial Strategies for an HIV Cure Meeting, 10–12 October 2018, Bethesda, MD, USA

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Abstract

The National Institute of Allergy and Infectious Diseases (NIAID) organised the Strategies for an HIV Cure 2018 meeting focused on research to develop innovative strategies for eradicating or achieving long-term remission of HIV infection. The purpose was to bring together researchers studying HIV persistence and cure strategies, including the six National Institutes of Health (NIH)-funded Martin Delaney Collaboratories for HIV Cure Research (MDCs), as well as industry and community partners, to share scientific results and stimulate active discussion among all stakeholders about the merits of various approaches under investigation. These discussions were intended to stimulate new collaborations and ideas for future research. The meeting covered a comprehensive range of topics spanning basic and translational research, drug discovery and development, and clinical research. Aside from the oral presentations described here, the meeting also included 130 poster presentations. Each of the three days of presentations is available for viewing via the NIH VideoCast website at: https://videocast.nih.gov/PastEvents.asp.

Day 1 keynote: pathways to sustained ART-free HIV remission

Anthony Fauci, director of NIAID, opened the meeting by outlining the results of several recent approaches to achieve sustained antiretroviral therapy (ART)-free remission of HIV infection when the current standard of care for most people is a one-pill-a-day regimen. He suggested that new therapeutic strategies should ultimately meet three criteria: (1) present low risk to individuals living with HIV; (2) be scalable; and (3) potentially induce an immune-mediated control of the virus. One approach involved the administration of a therapeutic vaccine to early-treated patients before interrupting ART in a double-blind placebo-controlled trial [1]. The vaccine was safe and well tolerated by all participants but was ineffective at delaying HIV rebound when compared to placebo control. The trial did, however, identify a number of participants in both the vaccine and placebo arms of the study who suppressed viral replication for months after interrupting treatment. This illustrates the importance of placebo control arms in clinical trials testing efficacy of HIV curative strategies as well as the prevalence of individuals capable of extended post-treatment control of the virus in the absence of an intervention.

Another alternative therapeutic approach is the passive administration of broadly neutralising monoclonal antibodies (bNAbs). Central to this approach is the utilisation of optimised antibodies with a longer half-life in the body and combining multiple bNAbs to combat pre-existing or newly developing HIV resistance. In a recent study involving non-human primates conducted by Malcolm Martin and Michel Nussenzweig, two bNAbs (10-1074 and 3BNC117) were passively transferred to animals 3 days after infection [2]. Significant control of viral replication was observed in 10 of 13 animals for over 2 years. Promising results from these approaches are paving the way to alternative, long-lasting (6 months or more) treatment options, potentially replacing daily medications for some patients in the future.

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Dr Fauci also provided an update on interventions targeting the integrin $\alpha4\beta7$. The NIH is conducting an open-label human clinical trial testing the safety and efficacy of blocking integrin $\alpha4\beta7$ in individuals with chronic HIV infection with the monoclonal antibody vedolizumab (ClinicalTrials.gov Identifier: NCT02788175). As part of the trial design, ART was discontinued. In contrast to prior experiments conducted in non-human primates (NHP) [3], the results from this trial were negative and may be explained by differences in the anti-$\alpha4\beta7$ antibody, how the trial was designed, or differences between NHP and human hosts. Further pursuit of this approach will require a larger, placebo-controlled trial.

The Martin Delaney Collaboratories for HIV Cure Research

The Martin Delaney Collaboratories for HIV Cure Research (MDC) programme is co-funded by NIAID, the National Institute on Drug Abuse (NIDA), the National Institute of Mental Health (NIMH) and the National Institute of Neurological Disorders and Stroke (NINDS). The programme is a research partnership between academia, government, industry, and community to conduct basic, translational, and clinical research towards a cure for HIV infection. Unlike many NIH grant programmes, the research foci of the MDCs are dynamic and designed to adapt quickly to advances in the field. The six collaboratories recently completed their second year of a 5-year funding term. The principal investigators for each MDC gave brief updates on their current areas of research focus and highlighted research that will be described in more detail under the subsequent sessions of the meeting.

I4C: Combined Immunological Approaches to Cure HIV-1

Dan Barouch provided research highlights for the Immunotherapy for Cure (I4C) MDC, based at the Beth Israel Deaconess Medical Center. The goal of I4C is to evaluate active and passive immunological strategies to target the viral reservoir in SHIV-infected rhesus monkeys and HIV-infected humans. Seven I4C members presented either oral or poster presentations over the course of the meeting. Dr Barouch described I4C's overall strategy of rapid elimination of virally infected cells using broadly neutralising antibodies (bNAbs) and latency reversal combined with long-term immune control of residual virally infected cells using therapeutic

vaccines to augment immune surveillance. He noted that early-phase clinical trials are planned to test dendritic cell-based therapeutic vaccines or bNAb combinations, and that additional studies will be planned, combining therapeutic vaccines or bNAbs with immunomodulators. The I4C MDC has a community engagement programme chaired by Charles Christen. Members have been recruited from Durban, Bangkok, Pittsburgh, and Boston to participate in meetings, host community education sessions, establish partnerships with local communities and provide feedback on protocol design.

BEAT-HIV: Delaney Collaboratory to Cure HIV-1 Infection by Combination Immunotherapy

Luis Montaner described that the BEAT-HIV MDC, based at the Wistar Institute, has grown from 35 to 68 members and includes 28 institutions and four industry partners. Five BEAT-HIV members gave oral presentations at the meeting, and 15 members had abstracts accepted, which he discussed briefly. A gene therapy trial in which HIV-specific chimeric antigen receptor (CAR) T cells are genetically modified to resist HIV infection is set to begin in November 2018. The BEAT-02 clinical study of IFNα2b combined with two bNAbs has been approved to move forward. Dr Montaner highlighted BEAT-HIV's new model for community engagement, called the Community Engagement Group (CEG), which is working on a community engagement video series to be completed in 2019. The group also held multiple workshops in 2018 and developed a position paper on ATI clinical trials.

BELIEVE: Bench-to-Bed Enhanced Lymphocyte Infusions to Engineer Viral Eradication

Douglas Nixon of the BELIEVE MDC, now based at Weill Cornell Medicine, described the collaboratory's approach to enhance immune strategies for HIV cure, specifically using immunomodulators, such as an IL-15 superagonist from partner NantKwest, and cell therapies to target viral reservoirs. The overall goal of BELIEVE is to first test strategies to enhance and improve immune responses to HIV using immunomodulators, targeting antibodies, or innate or adaptive cells as therapies; to then test combinations of these therapies; and to target these therapies to tissue sites where latent virus resides. He briefly discussed a myriad of highlights for year 2 including the approach to derive HIV-specific T cells with non-escaped epitope targeting HST-NEETs, which will be used in a Phase I study to evaluate safety, immunological and virological responses. He briefly touched on presentations by seven BELIEVE members and multiple posters that were accepted for the meeting. He concluded by presenting the members of the community advisory board (CAB) from Brazil, Canada, Mexico and Washington DC.

CARE: Collaboratory of AIDS Researchers for Eradication

David Margolis highlighted research from the CARE MDC, based at the University of North Carolina at Chapel Hill, over the last 2 years. The goals of CARE are to: (1) develop, test and validate novel latency-reversing agents (LRAs); (2) develop approaches to clear infected cells in the context of latency reversal; (3) develop, optimise, and validate assays to support *in vitro* and *in vivo* studies; and (4) to plan an iterative series of studies in animal models and humans to document latency reversal and viral clearance. His talk included a discussion of efforts to reverse latency, including the HDAC inhibitor vorinostat, STING agonists, and non-canonical NF-kB activation by SMAC mimetics to induce HIV transcription in latently infected cells. He also discussed efforts to combine LRAs with agents to target and clear latently infected cells, including dual affinity re-targeting (DART) molecules, the ADCC-mediating antibody DH677.4, and the bNAb VRC01-523LS. The community engagement activities of the CARE MDC included a number of capacity-building workshops and webinars in 2017 and 2018 in which many attendees discussed potential barriers to participation in trials, including compensation for participation, stigma and discrimination, and access to resources to facilitate participation.

defeatHIV: Delaney Cell and Genome Engineering Initiative

Keith Jerome discussed the unifying hypothesis of defeatHIV, based at the Fred Hutchinson Cancer Research Center, that cell and gene therapies represent a promising set of approaches for achieving sustained viral remission or cure of HIV infection. He provided updates for each of defeatHIV's research foci (RF). RF1 utilises SHIV-specific, bNAb-based CAR T cells engineered to resist HIV infection. Engraftment and persistence of low levels of CAR T cells has been demonstrated in 14 NHP using this approach. RF2 focuses on AAV vector-mediated delivery and expression of rhesus eCD4-Ig in NHPs for sustained viral clearance and ADCC of reservoir cells. RF3 involves combining therapeutic vaccination, protection of immune cells from HIV infection, and immune modulation. A study combining a DNA-based therapeutic vaccine with CCR5 knockout, GS-986 for latency reversal, and anti-PD-1 antibody as an immunomodulator is nearing completion in 24 animals. He ended his discussion with current CAB activities including community sessions with investigators and involvement with workshops on topics including inclusion of women in trials, acceptability of cell gene therapies balancing enthusiasm with unrealistic expectations in the community.

DARE: Delaney AIDS Research Enterprise to Cure HIV

Steven Deeks provided an update on the DARE Collaboratory, based at the University of California, San Francisco, which believes that the key to an effective HIV cure/remission strategy will be to generate a potent, sustained anti-HIV CD8-mediated immune response at the right place and at the right time. To this end, DARE has pivoted from an original focus on an SIV/CMV therapeutic vaccine to a conserved *gag/pol/nef* mRNA vaccine with partner CureVac. DARE is also exploring the effects of immune checkpoint blockade in NHP and in a cohort of cancer patients with HIV treated with pembrolizumab. Dr Deeks discussed two clinical trials in development: the first will use a therapeutic DNA/MVA vaccine with or without the anti-PD-1 mAb nivolumab to test for safety and immunogenicity, and the second will use the same IL-15 superagonist as BELIEVE (Nant-803) to test for safety, $CD8^+$ T cell density in lymph nodes, and B cell follicle disruption. The DARE CAB has had active outreach to the community in the last year, including multiple research forums and ongoing community engagement webinars, especially around the two developing clinical trials.

Session 1: finding the reservoir

The first session of the meeting was dedicated to identifying and characterising the persistent HIV reservoir in individuals on suppressive ART. The panelists described a range of approaches and technologies, including functional genomics, single-cell profiling, and imaging in either human infection or SIV-infected NHP models.

Ya-Chi Ho (Yale School of Medicine, USA) presented data building off observations from her lab and others that the persistent HIV reservoir consists largely of clonally expanded T cell populations with a common HIV integration site [4]. She discussed genomics approaches applied to the latent reservoir, including chromatin

accessibility assays and a new technique, SortSeq, which uses RNA probes to identify and isolate rare HIV RNA-containing T cells. The accessibility studies suggest that latent T cells exist in a state of deep latency that is resistant to transcriptional reactivation. SortSeq analysis demonstrated that HIV frequently integrates into housekeeping and cancer-related genes, and that RNA transcription driven by HIV LTRs can impact host-cell gene expression in many ways, including the generation of truncated host proteins, host/HIV fusion proteins, and antisense RNA. These gene products may cause cellular stress, even when ongoing HIV replication is fully suppressed. Furthermore, LTR activation of cellular oncogenes may drive proliferation of latent clones. These scenarios argue for development of drugs that target HIV-mediated transcription to stem expansion of the latent reservoir and modulate pathogenesis during suppressive ART.

Edward Browne (University of North Carolina at Chapel Hill, USA) reported on his lab's single-cell RNA sequencing (scRNASeq) approaches to characterise transcriptional profiles of latently infected T cells, with the goal of identifying host factors that impact latency [5]. His lab uses a primary CD4 T cell model incorporating an HIV-eGFP reporter virus and long-term culture on feeder cells to induce latency. Reactivation results in a high percentage of GFP-positive cells, which return to a latent state when re-cultured on the feeder cells. The team explored transcriptional profiles of cells either entering into or reactivating from HIV latency. scRNASeq revealed that latency could occur in diverse host-cell environments, most frequently in cells with naïve and central memory signatures with high proliferative capacity. Moreover, the analysis identified the transcriptional activator GATA 3 as a candidate host latency-reversal factor. The approach, therefore, has the potential to identify novel cellular targets for modulation of the latent reservoir.

Nicholas Chomont (University of Montreal, Canada) presented new data using the TILDA (Tat/rev induced limiting dilution assay) reservoir measurement method developed in his lab [6]. TILDA quantifies multiply spliced HIV RNAs (msRNA) in CD4 T cells, a sensitive measure of cells harbouring transcriptionally active proviruses. He compared TILDA measurements in matched lymph node (LN) and PBMC from individuals on suppressive ART with and without *in vitro* activation, thereby measuring both the 'active' and the inducible 'total' reservoir. Interestingly, LN T cells showed a significantly higher basal level of RNA expression than PBMC, while the total signal in activated LN samples was similar to PBMC. These results suggest that, in LN, the majority of the reservoir is transcriptionally active and may be the source of early rebounding HIV following treatment interruption. Further characterisation of reservoir cells by flow cytometry confirmed the presence of 'active' reservoir cells in the tissues and identified TIGIT, PD-1 and $\alpha 4\beta 1$ as potential markers of the reservoir in lymphoid tissues. Additional validation of these markers may assist in the ongoing search for new biomarkers of the reservoir.

Jacob Estes (Oregon Health and Science University, USA) presented imaging-based analyses of the SIV reservoir in NHP models using the fluorescence *in situ* hybridisation (FISH) techniques, RNAScope and DNAScope [7]. His lab and others have demonstrated that the vast majority of HIV RNA-expressing cells reside in lymphoid tissues, in both untreated and ART-suppressed infection. Combining the FISH techniques revealed that proviral DNA-positive, RNA-negative cells, presumably representing the latent SIV reservoir, also reside primarily in lymphoid tissues in ART-suppressed monkeys. Further combination of Scope with immunofluorescent staining identified the infected cell subtypes, including the accumulation of infected, IL-10-positive follicular helper T cells (Tfh) within lymphoid follicles. A limitation of these studies is that the DNA FISH probes detect both intact and defective proviral sequences, thus overestimating the size of the replication-competent reservoir. To overcome this, the lab developed RNA and DNA probes specific for regions of the genome that are most frequently deleted in defective proviruses. These approaches, termed BASEscope, can more accurately identify infected cells that harbour intact genomes in the tissue compartment, an important next-generation approach to imaging the active and latent reservoir [8].

Thomas Hope (Northwestern University, USA) presented his group's 'imaging toolbox,' a suite of novel probes and technologies for multi-scale imaging. They have developed SIV reporter constructs that enable detection of infection, first by bioluminescence to home in on the tissue of interest, followed by dissection and fluorescence imaging to identify individual infected cells [9]. When combined with correlative electron tomography it is possible to visualise individual virions – an impressive range of resolution from whole animal to single virion imaging [10]. Dr Hope also presented PET/MRI imaging of antibody distribution in macaques, revealing the dynamic distribution of monoclonal antibodies (mAb) following IV administration [11]. These studies inform a broad range of mAb trials currently being implemented for HIV prevention or cure. He finished with some provocative data demonstrating the feasibility of directly imaging viral rebound following ART interruption in infected macaques. While challenging, these approaches hold promise to identify the anatomical sites and cell populations responsible for initiating rebound, a key target for intervention strategies aimed at curing infection.

The panel discussion following the session focused primarily on the central question: what and where is the reservoir? While much of current cure research is focused on the transcriptionally inactive, replication-competent provirus pool residing in T cell populations, it is still unclear what role the 'active' reservoir expressing defective and intact HIV plays in ongoing pathogenesis during therapy and rebound following ART interruption. Additionally, the dynamic nature of expanded latent populations, variations in the timing of initial ART, and the duration of suppressive therapy make it difficult to draw conclusions from different studies.

Session 2: quantifying the reservoir

Robert Siliciano (Johns Hopkins University, USA) discussed the critical need for accurate, scalable HIV reservoir assays to evaluate HIV cure strategies. Current standard PCR assays may not detect effective interventions because the vast majority of HIV genomes are defective. The quantitative viral outgrowth assay (QVOA) is the current gold standard assay to determine persistent HIV burden, but many intact HIV genomes are not induced following a single round of stimulation in the QVOA assay. These proviruses could theoretically be a source of HIV rebound in infected individuals. In response to these problems, Dr Siliciano and his laboratory developed a new assay, the intact proviral DNA assay (IPDA). This assay takes advantage of advancements in droplet digital PCR, where a single HIV genome can be subjected to PCR within an oil droplet. Two amplicons, one overlapping the packaging signal (ψ) and one overlapping the Rev-responsive element (RRE) in the HIV envelope gene, can accurately distinguish between defective and intact proviral HIV genomes. IPDA analysis of patient samples indicated that 97% of genomes were defective, and the frequency of intact HIV genomes was approximately 100 per 1 million CD4+ T cells, consistent with more labour-intensive methods utilising long distance PCR. By simultaneously excluding defective proviruses and detecting all intact HIV genomes, the IPDA approach seems poised to replace QVOA as

the new gold-standard tool for measuring selective reductions in the HIV reservoir in future HIV cure studies.

Michael Busch (Vitalant Research Institute, USA, formerly the Blood Systems Research Institute) discussed the latest findings from RAVEN (Reservoir Assay Validation and Evaluation Network). The goal of RAVEN is to help advance and validate performance of HIV reservoir monitoring assays. In a recent study, traditional QVOA was compared to alternative assays measuring cell-associated RNA, cell-free RNA, HIV DNA, or p24 endpoints across different labs using blood samples from the same six volunteers. Key questions were as follows. How do these assays perform in different labs? Does cryopreservation affect the results? Are any of the alternative assays good proxies for traditional QVOA? The results indicated that freezing and thawing of samples does not significantly affect the reproducibility of QVOA. RNA-based endpoints result in modest to significantly higher measurements of HIV burden in the samples. A majority of this signal appeared early in cell culture and may represent defective genomes. The use of longitudinal sampling is therefore necessary to avoid overestimation of HIV burden compared with the traditional p24 ELISA method of QVOA detection. Ultrasensitive p24 assays were found to be unsuitable for direct measurements in plasma samples.

Elizabeth Anderson (HIV Dynamics and Replication Program, National Cancer Institute, USA) presented her work on detection of HIV DNA decay upon ART initiation. A great deal is known about how HIV RNA in plasma decays once patients start treatment. Likewise, it is well appreciated that HIV DNA can be detected for years even after plasma viraemia is undetectable, but the dynamics of HIV DNA decay immediately following ART initiation is poorly described. To address this question, a droplet digital multiplex PCR approach was used to quantify total HIV DNA in 10 participants. This involved the generation of six amplicons sampling portions of the HIV genome overlapping the LTR, *gag*, and *tat* regions. In contrast to plasma viraemia, which eventually declines 99.9% after ART initiation, during that same time, HIV DNA only declines 75%. After only 6 days on ART, plasma viraemia decayed 96% while, on average, HIV DNA only decayed 30%. This finding suggests that very few cells in the blood contribute to plasma viraemia. The contribution of lymph node cells harbouring HIV DNA to plasma viraemia is currently under investigation.

Zabrina Brumme (Simon Fraser University, Canada) shared her recent work on a phylogenetic approach to estimating the age of latent HIV sequences. She highlighted a fundamental difference in the dynamics of HIV in the plasma versus the latent reservoir, which is established within hours or days after infection and can persist in an individual for many years. During this time, it is unclear whether the reservoir represents a cumulative archive of HIV evolution within a host or if dynamic processes including clonal expansion disproportionately influence the genetic composition of HIV. To address this question, Dr Brumme has developed a model for inferring the original integration dates of individual latent HIV sequences [12]. This model is dependent on samples collected on known dates prior to initiation of ART and is based on sub-genomic sequencing. A linear regression is created based on the sequences of these pre-ART samples that relates genetic distance to time. Using this approach, the integration date of a given HIV provirus in an individual can be inferred solely from its sequence. Two example cases were presented. In one individual, the reservoir was sampled over 20 years and contained sequences at later time points that had disappeared from circulation in the plasma many years prior. In contrast, a second individual's reservoir contained sequences more closely related to the sampling times near the initiation of ART. The differences between these two examples highlights the variability among individuals. The study of more participants will be required to better understand how the composition and dynamics of the latent reservoir varies across a population.

Brandon Keele (AIDS and Cancer Virus Program, National Cancer Institute, USA) presented his work using a barcoded virus (SIVmac239M) model in NHP to assess reductions in the HIV reservoir by measuring the reactivation rate. The reactivation rate is defined as the time it takes for one cell to become activated and produce progeny, ultimately resulting in viral rebound. An advantage of the barcoded system in NHP is the ability to accommodate a large reservoir because individual reactivation rates can be measured. To date, a cumulative total of 120 rhesus macaques have been infected with the barcoded viruses. The vast majority of these barcodes are functional and can be found *in vivo,* indicating that the barcode does not affect acquisition, replication or distribution in the animal. The near full-length sequencing of the barcoded viruses indicated that approximately 80% of viral sequences were intact, likely to be reflecting, in part, the early timing of ART. This model system could provide a valuable tool for evaluating novel curative intervention strategies.

Day 2 keynote: CD4-mimetic compounds. Sensitisation of HIV-1 to sub-dominant antibody responses

Joseph Sodroski (Dana-Farber Cancer Institute, USA) opened the second day with a keynote presentation on a novel approach that could potentially be used to aid clearance of reservoir cells by the immune system. Building on evidence that HIV-1 gp120 envelope (Env) trimers convert from closed conformations to more open conformations upon binding to CD4, Dr Sodroski and his collaborators are developing CD4-mimetic compounds that bind to a highly conserved pocket on Env and trigger its conversion to an open conformation [13]. These compounds sensitise primary HIV-1 isolates to neutralisation and ADCC by antibodies targeting highly conserved Env epitopes that are elicited at high titres during natural HIV-1 infection [14–16]. While these antibodies only weakly neutralise when Env is in the closed conformation, they potently neutralise and trigger antibody-dependent cellular cytotoxicity (ADCC) when Env is opened by CD4-mimetics [17]. One mimetic compound, BMN-III-170, has demonstrated acceptable safety and toxicology in rhesus monkeys when administered subcutaneously at 24 mg/kg. A single injection achieves plasma concentrations above the IC_{50} for 12–24 hours, indicating adequate pharmacokinetics for efficacy testing in SHIV infection models.

Session 3: overcoming latency

The overarching goal of this session was to highlight cutting-edge research in HIV latency-reversal strategies. The science presented began with early *in vitro* and *ex vivo* studies, followed by animal studies in humanised mice and NHP, and ended with ongoing clinical studies.

Amanda Macedo (George Washington University, USA) presented her studies using dual TLR2/TLR7 agonists as HIV latency-reversing agents (LRAs) [18]. Toll-like receptors (TLRs) play major roles in innate and adaptive immunity. Given the different pathways that TLRs regulate, Dr Macedo hypothesised that agonists that targeted multiple TLRs with one molecule might promote more potent immune responses in response to therapeutic interventions. Dr Macedo's study investigated the activity of a dual TLR2/7 agonist, CL413, in the reactivation of latent HIV. First, her team showed that this TLR2/7 agonist can reactivate primary CD4 T cells in a Tcm cell model. Next, they demonstrated that the dual

TLR2/7 agonists induced the secretion of TNF-α from PBMCs, and that TNF-α is the cytokine responsible for the paracrine effect on viral reactivation. These data suggest that complementary mechanisms may favour the reactivation and clearance of latent reservoirs and that CL413 or other dual TLR2/7 agonists warrant further testing in HIV eradication studies.

R. Brad Jones (Weill Cornell Medicine, USA) discussed his work showing that BCL-2 antagonism enables CTL-mediated elimination of CD4 reservoirs *ex vivo*. Previous work by Huang *et al.* [19] demonstrated that latent HIV reservoirs exhibit inherent resistance to elimination by CD8 T cells despite the use of highly potent LRAs. Dr Jones hypothesised that this resistance to elimination may be overcome by other factors. Building on prior work by Cummins *et al.* [20], Dr Jones' group investigated the activity of BCL-2/BCL-xL inhibitors, first in a primary cell latency model and then in *ex vivo* participant CD4 T cells. They demonstrated that these BCL-2/BCL-xL antagonists reduced HIV DNA and the infectious reservoir (as measured by QVOA) in a primary cell latency model; however, these reductions were not recapitulated in the *ex vivo* participant CD4 T cells. The group next combined the BCL-2/BCL-xL inhibitor with anti-CD3/anti-CD28 (for latency reversal) and HIV-specific CTL effector cells and saw reductions in the *ex vivo* donor cells. These data indicate that BCL-2/BCL-xL antagonism may be effective in facilitating CTL-mediated elimination of reservoirs in future studies.

J. Victor Garcia (University of North Carolina at Chapel Hill, USA) described studies on the systemic *in vivo* induction of the latent HIV reservoir via a non-canonical NF-kB signalling pathway. His group hypothesised that induction of HIV in resting, latently infected CD4 T cells *in vivo* using a SMAC mimetic might result in reservoir reduction. Dr Garcia and his collaborators focused on the molecule AZD5582, a synthetic inhibitor of apoptosis that has been previously shown to induce HIV RNA expression in resting CD4 T cells from ART-suppressed donors, with limited pleiotropic effects. Utilising the BLT humanised mouse model, the team demonstrated robust induction of plasma viraemia in ART-suppressed BLT mice upon AZD5582 administration, as well as increased HIV RNA in the female reproductive tract, peripheral blood and brain. Importantly, the team was able to demonstrate robust induction of HIV RNA specifically in resting CD4 T cells isolated from various tissues, including liver, bone marrow, lymph nodes, and thymus. In assessing off-target toxicities, they showed that AZD5582 treatment resulted only in transient toxicity and no inductions of cytokine or chemokine expression. These results represent the first *in vivo* evidence of systemic HIV latency reversal in resting CD4 T cells in tissues, without overt toxicity.

Building on the SMAC mimetic studies in mice, Ann Chahroudi (Emory University, USA) presented an NHP study investigating SIV latency reactivation upon treatment with AZD5582. Rhesus macaques were infected with SIVmac239 and suppressed on ART for over a year. Those in the experimental group received weekly AZD5582 infusions and were compared to control animals. The experimental group demonstrated various patterns of virus reactivation over 10 infusions of AZD5582. These animals exhibited increased cell-associated RNA and DNA compared to controls. Importantly, AZD5582 treatment did not result in generalised CD4 T cell activation. Notably, SIV-specific T cell responses were not impaired with AZD5582, which has been a concern with other LRAs. Altogether, these data indicated that targeting the non-canonical NF-kB pathway with SMAC mimetics is a novel and effective strategy for reversing HIV/SIV latency *in vivo*.

Following the prior presentations on studies in preclinical cell and animal models, Session 3 culminated with a presentation by Devi SenGupta from Gilead Sciences on clinical studies with TLR7 agonists. She provided an introduction to Gilead's TLR7 agonist, GS-9620, known as vesatolimod [21]. She described a vesatolimod dose-escalation study (NCT02858401) in individuals with chronic HIV infection that was suppressed by ART, with the primary objective to assess safety and tolerability. A second study (NCT03060447) will be investigating the effect of vesatolimod in virological controllers in the context of an analytical treatment interruption (ATI). In addition to studying safety and PK/PD, this study will assess changes in the reservoir and viral rebound in a 24-week ATI. Given preclinical NHP proof-of-concept studies on combinatorial approaches with bNAbs and TLR7 agonists [22], Gilead will be performing a first-in-human study of the bNAb, GS-9722 (a derivative of PGT-121). Additional studies are planned that would combine GS-9620 with GS-9722. In summary, there is great anticipation for the results of these and future combination studies using TLR7 agonists in human clinical trials.

Session 4: killing reservoir cells

The goal of strategies to eliminate HIV rather than suppress it long term is the killing of latently infected cells. The presentations in this session explored how this might be accomplished by immune effectors, i.e. antibodies and/or cytotoxic cells (NK or T). In animal model or human studies where both antibodies and cytotoxic cells are present, direct measurement of effects on reservoir size are necessary to distinguish killing of infected cells from other mechanisms that might affect outcome measurements in a similar way.

Matthew Gardner (Scripps Research Institute, USA) presented studies on eCD4-Ig, an antibody-like molecule that is a broadly neutralising, potent inhibitor of HIV [23-24]. The IgG2 form, itself incapable of mediating ADCC in the presence of NK cells, induced changes in cell surface HIV envelope that facilitated the ADCC activities of non-neutralising V3-loop and CD4i antibodies. The IgG1 form, capable of ADCC activity, enhanced ADCC activity of serum from infected persons and mediated ADCC against latently infected cell lines in which HIV was reactivated. In NHP studies, AAV-expressed eCD4-Ig maintained post-treatment viral control in 50% of SHIV-infected, ART-treated animals.

Guido Ferrari (Duke University, USA) introduced his talk by asking the question 'how many monoclonal antibodies are required to target cells infected with isolates from the latent reservoir?' He then discussed his studies using HIV-specific monoclonal antibodies that mediate ADCC [25]. His research has determined that cell surface-expressed HIV envelope is recognised differently by antibodies capable of mediating ADCC. The non-neutralising mAb A32 preferentially binds to, and mediates, ADCC against CD4-positive cells, while a panel of bNAbs preferentially bind to, and mediate, ADCC against cells that have downregulated CD4. An extensive panel of mAbs was tested for ADCC activity, alone and in combination. It was found that a combination of at least three mAbs that include both non-neutralising and broadly neutralising antibodies are needed to efficiently recognise and eliminate cells infected by diverse isolates of HIV.

The next two talks in this session discussed the development of animal models to test antibody and cell-based approaches for reservoir reduction or elimination. Harris Goldstein (Albert Einstein College of Medicine, USA) described a new humanised mouse model engrafted with well characterised PBMCs from persons with HIV infection suppressed by ART [26]. The novelty of the model is the use of an intrasplenic injection of cells, which provides a confined lymphatic environment that allows for the development

of robust viraemia by 2 weeks post-injection. Experiments with FcR-null mice and the mAb 10-1074 supported a role for Fc-mediated pathways such as ADCC in the delayed onset of viraemia and emergence of new viral sequences in 10-1074-treated mice. Dissection of the mechanisms involved will be obtained by depletion of cell subsets prior to engraftment.

Christopher Peterson (Fred Hutchinson Cancer Research Center, USA) illustrated the additional information that can be garnered from the use of an immunocompetent large-animal model. He described experiments in which virus-specific, stem cell-derived CAR T cells persisted and reduced infection in SHIV-infected animals. He demonstrated robust trafficking of the CAR-modified cells to LN germinal centres using immunohistochemical methods and identified individual lineages (CD4 and CD8 T cells, B cells, macrophages, and follicular dendritic cells) among the modified cells in the tissue using multiplex imaging. The importance of these studies is the demonstration that the stem cell-derived CAR cells are capable of long-term engraftment and traffic to locations in the body where they are needed to exert killing and immune surveillance [27].

The remaining presentations in the session focused on CAR T cells. James Riley (University of Pennsylvania, USA) discussed his work with CD4-based CAR T cells. He started with a CAR design that had shown no significant antiviral effects in clinical trials and then optimised a number of vector elements (backbone, promotor, targeting element, transmembrane and signalling domains). The re-engineered CAR provided a 50-fold improvement in anti-HIV activity in an *in vitro* system and controlled HIV replication better than the first-generation CAR in a humanised mouse model [28]. A clinical trial is planned that will test the latest CAR design for its ability to control HIV replication in the absence of ART.

The session closed with a presentation by Thor Wagner (Seattle Children's Hospital, USA) describing the first large-animal trial of a gene-edited CAR T cell product. Cells were 'protected' from infection by CCR5 disruption and engineered to express broadly neutralising HIV-specific antibody CARs. Cells were successfully manufactured and injected into NHP. Engineered cells were detected by flow cytometry at low levels but did not persist for extended periods of time. Efforts are underway to optimise engraftment, trafficking and persistence.

Session 5: immunomodulation and immunological control of viraemia

Afam Okoye (Oregon Health & Science University, USA) presented data that depleting CD8 T cells in SIV-infected rhesus macaques using a CD8 beta-specific antibody neither induced viral blips during antiretroviral treatment nor impacted time to viral rebound or viral reactivation rates upon cART cessation. However, Dr Okoye also demonstrated that HIV-1-specific CD8 T cells expand during viral rebound, limit viral replication in lymph nodes, and reduce plasma setpoint, thus supporting the concept that T cell-based therapeutic vaccines might be efficacious if they could elicit earlier, more potent, control of HIV-1 spread.

Rachel Rutishauser (University of California, San Francisco, USA) demonstrated that HIV-1-specific CD8 T cells from natural HIV-1 controllers exhibit much greater proliferative capacity *in vitro* as compared with T cells from ART-treated or viraemic individuals. She discovered that controller T cells express elevated levels of TCF-1, a Wnt-signaling transcription factor upregulated in T cells with stem cell-like memory properties and previously associated with improved proliferative/regenerative capacity. She demonstrated that depleting TCF-1 reduced the proliferative capacity of human CD8 T cells *in vitro,* and that the levels of TCF-1 expression in HIV-1-specific CD8 T cells correlated inversely with viral loads in viraemic individuals. These findings suggest one aim of therapeutic vaccine and cure strategies should be to expand HIV-1-specific CD8 T cells with high TCF-1 expression.

Sandra Dross (University of Washington, USA) discussed myeloid-derived suppressor cells (MDSC). The frequencies of these cells correlate with viral loads during HIV and SIV infection and they suppress T cell proliferation and cytokine production *in vitro*. Using rhesus macaques infected with SIV and treated with ART, Dr Dross demonstrated that MDSC numbers increase significantly upon treatment interruption [29]. When the macaques were vaccinated therapeutically with Gag conserved elements while on ART, the levels of MDSC at vaccination inversely correlated with peak vaccine-specific ELISPOT responses. These results suggest that MDSC may mask therapeutic vaccine responses at the time of intervention or during treatment interruption, thus providing strong rationale for studies aimed at depleting MDSC in conjunction with HIV remission strategies.

James Whitney (Beth Israel Deaconess Medical Center, USA) discussed efforts to develop combination immunotherapies for the induction of durable HIV remission. As a model, rhesus macaques were intrarectally infected with SIVmac251 and treated with ART 65 days later. Once stably suppressed, groups of animals received either five doses of a TLR7 agonist, six doses of an anti-PD-L1 antibody, both in combination, or placebo. Sporadic transient inductions of plasma viraemia were observed after TLR7 and/or anti-PD-L1 treatments. Six of 16 animals in the active treatment groups, but none of the four animals in the placebo group, displayed durable viral remission after cessation of ART. Animals in the active treatment groups also displayed increased frequencies of CXCR5- and CXCR3-positive CD8 memory T cells in lymph nodes and reductions in cell-associated SIV DNA in lymph nodes and colorectal mucosa. These results provide a signal of possible efficacy for this combination remission strategy in a stringent animal model and indicate that high-intensity dosing of TLR7 agonist and anti-PD-L1 immunotherapy in combination can be delivered safely to SIV-infected ART-treated macaques.

Dan Barouch (Beth Israel Deaconess Medical Center, USA) described a study in which rhesus macaques were infected intrarectally with SHIV-SF162P3, treated with ART for 96 weeks beginning at day 7 post-infection, and then treated with either 10 doses of the TLR7 agonist GS-9620, five doses of the V3 glycan-directed bNAb PGT121, both TLR7 agonist and PGT121 in combination, or placebo [22]. The groups receiving GS-9620 showed increased CD4 T cell and NK cell activation at day 1 after treatment. The group receiving the combination of GS-9620 and PGT121 showed significantly reduced lymph node SIV DNA and delayed time to viral rebound when ART was discontinued 16 weeks after the last treatment. The PGT121-only group also showed significantly delayed viral rebound, though to a lesser degree than the combination group. Both PGT121-treated groups also showed decreased viral load setpoints following ART discontinuation. Five of 11 animals receiving the GS-9620/PGT121 combination therapy showed no rebound for 6 months after ART discontinuation, with no evidence for residual reservoir as indicated by adoptive transfer and CD8 depletion studies. The data suggest that combining bNAbs with an innate immune stimulant can effectively target viral reservoirs while animals are on ART, perhaps by inducing envelope expression on reservoir cells and thereby enhancing their recognition by bNAb and clearance by activated NK cells.

Marina Caskey (Rockefeller University, USA) described a human clinical trial evaluating the effects of a two-bNAb combination

in individuals with HIV on ART [30]. Participants were selected based on a neutralisation assay that determined whether HIV-1 cultured from their blood was sensitive to the bNAbs 3BNC117 and 10-1074, which were previously shown to be safe and well-tolerated in humans when administered as monotherapy. Fifteen participants were enrolled and treated with a combination of the two bNAbs, which were administered at 30 mg/kg on weeks 0, 3, and 6 after ART discontinuation. The median time to viral rebound was 15 weeks overall. Earlier rebound was associated with pre-existing resistance to at least one of the bNAbs when reservoirs were examined intensively and/or to incomplete viral control while on ART. Late rebound occurred after one or both bNAbs were reduced to subtherapeutic levels. The median time to rebound for the 11 late rebounders was 21 weeks, excluding two participants who maintained viral suppression until the end of follow-up. There was no evidence for development of *de novo* resistance while both bNAbs were maintained above 10 µg/mL in plasma. The participants' reservoirs consisted in large part of expanded clones whose frequencies changed dynamically during bNAb therapy, although seemingly not due to pressure from the bNAbs. Reservoir size did not appear to change in magnitude during the combination bNAb therapy.

Day 3 keynote: silencing the HIV-1 reservoir

Day 3 began with a keynote address by Susana Valente (Scripps Research Institute, USA) asking the question 'can a functional cure be achieved by silencing transcription from the viral reservoir?' Her research has investigated the possibility that a Tat inhibitor could induce a durable state of latency ('deep' latency). The approach, referred to as 'block-and-lock', would provide long-lived (possibly permanent) suppression of virus expression in contrast to the alternative cure approach which involves the elimination of HIV through latent virus activation and subsequent destruction of virus-infected cells. Several years ago, Dr Valente and her team discovered that an analogue of the natural product cortistatin A, didehydrocortistatin A (dCA), is a potent inhibitor of Tat (IC_{50} in the nanomolar range). dCA appears to be non-toxic in commonly used cell-line models of HIV infection, in CD4+ cells isolated from aviraemic individuals with HIV, and in HIV-infected BLT mice, while the parent compound has been reported by other investigators to have anti-proliferative activity when tested against unrelated cell lines (HUVEC and AML-derived). dCA mediates epigenetic silencing by causing a block at nucleosome-1 during HIV transcription that impedes the movement of RNA polymerase II to the HIV-1 promotor and thus blocks elongation of viral RNA. In an *in vitro* system, dCA prevents viral rebound from CD4+ T cells after treatment interruption, even in the presence of strong cellular activation. In HIV-infected BLT mice, it reduces viral mRNA in tissues and delays and reduces viral rebound upon antiretroviral treatment interruption [31]. Some questions remain regarding the potential of this approach and include: can a permanent block be maintained in the absence of ARVs? If a permanent block cannot be achieved, how long could a block be maintained? What would the long-term toxicities of treatment with dCA be? Can related analogues be derived that are more easily synthesised? And can other Tat inhibitors with different structures be discovered and tested for activity?

Session 6: clinical trials and community engagement

Deborah Persaud (Johns Hopkins University, USA) reviewed outcomes from a meeting on the 'Framework for Initiating Pediatric Studies of HIV Cure Interventions', sponsored by the NIH and the Forum for Collaborative Research. She commented that despite laws requiring that drugs be developed for infants/children as well as adults if they also have the disease, only nine of 22 (41%) FDA-approved and marketed antiretrovirals have approved formulations for infants <3 months of age.

Infants, children and adolescents with HIV have fundamental immune differences as compared to adults. This provides justification for testing low-risk remission or cure interventions in paediatric populations in parallel with adult populations. In newborns, timing of HIV exposure is known and is followed by immediate ART initiation and testing, which results in smaller reservoirs, less viral diversity, and preserved host immunity. Compared to adults, infants have: a higher percentage of naïve $CD4^+$ T cells; more T_{reg} cells, higher $CXCL8^+$ CD8 T cells; lower cytokine levels; and higher B cell counts. Post-treatment control of viraemia in the absence of ART has been demonstrated in the Mississippi baby [32], the French adolescent [33] and the South African child [34]. Studies in infant macaques demonstrated that early short-term treatment with human bNAbs halted SHIV infection [35]. Of interventions targeting remission in adults, only early ART ± bNAb (VRC01) and cord blood transplant of CCR5delta32 cells are currently being studied in paediatric populations. Additional studies are planned in paediatric populations to test early ART with combination bNAbs, and ART plus a therapeutic vaccine.

Boris Juelg (Ragon Institute, USA) discussed outcomes from the 'Consensus Workshop on Analytical Treatment Interruption in HIV Cure Trials', sponsored by the Ragon Institute, which included participants from academia, industry, funding institutions, the US FDA, ethicists and the community. The goals of the meeting were to reach consensus where possible on inclusion and ART restart criteria for ATI studies. There was agreement that early phase ATI studies should be conducted in the healthiest ART-suppressed population possible and should be designed to maximise the utility of the study while minimising risk and inconvenience to participants. Reservoir expansion and CD4 cell count declines during ATI have been transient, and emergence of resistance to pre-ATI ART or retroviral syndrome has not been realised [36-37]. However, the potential risk of HIV transmission to sexual partners during the ATI remains a concern. Although consensus was not reached on many issues, at least 50% of the participants supported excluding individuals with a CD4 cell count nadir of <200 cells/mm^3, a CD4 cell count of <500 cells/mm^3 (although, an additional ~38% of participants supported excluding participants with a CD4 cell count of <350 cells/mm^3), or a history of, or risk for significant end-organ disease. The paediatricians agreed that ATIs should not be attempted in individuals <2 years of age because their immune systems have not yet had a chance to mature. During ATIs participants should be monitored weekly for clinical symptoms, viral load, and CD4 cell count for 12 weeks and, thereafter, every other week. ART should be restarted: upon the participant's request, as needed for clinical care, upon a CD4 cell count <350 cells/mm^3 or <15%, or upon a viral load of $>10^5$ RNA copies/mL (or upon meeting a lower protocol-defined viral load criteria). Viral load criteria and duration of viraemia will depend on whether the desired outcome of the intervention is reservoir eradication (no viral rebound) or immune enhancement leading to control of viraemia (which may require several weeks of transient, high-level viraemia). Investigators will need to justify the endpoint selected, and continued dialogue will be necessary as new data become available.

Karine Dubé (University of North Carolina at Chapel Hill, USA) presented results of a survey conducted among US adults living with HIV concerning perspectives on target product profiles for HIV cure regimens and factors affecting the likelihood of enrolment

in HIV 'cure' trials, with a goal of identifying ways to increase the proportion of cis and trans women participating in trials. Perceived motivators for both genders, but more common in women, included: feeling good about helping others with HIV; advancing 'cure' research; access to doctors/researchers; gaining special knowledge about HIV and their health; support from their family and friends; financial compensation; and reimbursement for transportation. Perceived risks that would prevent participation in research included: risk of dementia or problems thinking; lasting pain or discomfort; developing resistant virus; changes to the immune system that could put them at risk; risk of losing health insurance; being treated poorly by study staff; and fear of transmitting HIV to others. Women were more concerned about: the risk of transmitting HIV to others; problems with bones or muscles; the need to delay having children; and temporary physical pain or discomfort from study procedures. Respondents considered dosing every 6 months or less frequently (long-acting ART) to be an improvement relative to one pill, once a day, and 73% of respondents were willing to try a strategy for sustained viral remission to avoid the long-term consequences of ART. This study and others highlight how social/behavioural scientists who focus on acceptability can enhance the research conducted by biomedical personnel who focus on safety and efficacy.

Sara Gianella Weibel (University of California, San Diego, USA) reported on 'The Last Gift: Performing HIV Cure Research at the End of Life', an innovative approach initiated in July 2017 to assess the HIV reservoir in human tissues that have been impossible to collect in clinical trials. The programme enrols individuals living with HIV and with a terminal illness, conducts qualitative interviews with participants and their next of kin, collects blood hours before death to assess viral load and ART concentrations, and completes an autopsy, collecting and freezing tissues within 6 hours of death to optimally preserve HIV DNA and RNA. Tissues are then assayed using digital PCR to evaluate the size and diversity of the HIV reservoirs. The survey has identified societal and psychological benefits to the participant, with participants reporting a deep sense of purpose and pride in being an integral part of the HIV cure research process [38,39]. Many of the community advisory board members who assisted in developing the informed consent form and the next of kin survey stated that they look forward to participating in the study when their time comes. The study team welcomes collaborations to generate the maximum scientific information from the generous tissue donations from these altruistic study participants.

Session 7: viral rebound and post-treatment control

Jintanat Ananworanich (Military HIV Research Program, USA) opened the session with results from three clinical ATI studies associated with the RV254 cohort of early-treated individuals. A total of 40 participants were enrolled in trials designed to test the safety and impact of three different interventions on time to viral rebound after stopping ART: (1) very early ART (Fiebig stage I; RV411) [40]; (2) an LRA (vorinostat; RV409); or (3) bNAb administration (VRC01; RV397). In each case, most participants experienced rapid viral rebound with the expected continuous rise in viral load, but in five of the 40 individuals, a different pattern of viral load rise and fall was observed, with variation in the time to viral load rebound. The importance of analysing tissue reservoirs to identify possible sources or predictors of rebound was examined. Results from optional tissue biopsy procedures showed little evidence for viraemia in cerebrospinal fluid, but viral RNA was observed in gut and lymph node tissue samples before, during and after brief ATI in 30–50% of participants. To alleviate the burden of frequent monitoring (identified in one study as the primary deterrent to participating in ATI studies), future studies should consider reducing monitoring to every 1–2 weeks and prioritise invasive procedures to collect only the most informative tissue samples. Surprisingly, some adverse events were reported during ATI even when virus was undetectable, although none met the criteria for acute retroviral syndrome. In three of 12 participants, viral RNA was detected in anal secretions, and two of the three individuals had concomitant STIs detected, highlighting the importance of adequately informing participants about the potential risks of transmission during clinical trials involving ATI.

Katharine Bar (University of Pennsylvania, USA) presented preliminary testing of a new PhenoSense assay to identify resistance to a panel of bNAbs in samples from participants living with HIV. In recent clinical trials testing whether bNAb interventions could control viraemia in the absence of ART, the extent of pre-existing virus resistance was found to be a critical barrier to bNAb efficacy [30,41]. Results from the new assay, developed by Monogram Biosciences, correlated with findings from prior trials, and showed similar results using blood cells and plasma. The assay is pending CLIA certification and will be validated in conjunction with several upcoming clinical trials testing bNAb interventions for viral control after treatment interruption. If successful, the assay will be an important tool for use in screening trial participants for pre-existing bNAb resistance upon enrolment in future clinical trials.

Sarah Joseph (University of North Carolina at Chapel Hill, USA) presented analyses of samples from women in the CAPRISA cohort that were aimed at defining when the latent HIV reservoir is seeded. Her studies compared HIV sequences from multiple time points before ART initiation to outgrowth virus from blood samples taken after 4–5 years of viral suppression. In most women, the majority of the replication-competent reservoir matched HIV sequences from the year just prior to the initiation of treatment; whereas, only a small fraction of outgrowth sequences matched samples from early after primary infection. Further modelling studies were consistent with the idea that most HIV reservoir cells are relatively short-lived, but that ART initiation triggers the formation of long-lived HIV reservoirs. If true, manipulation of T cells at the time of ART initiation may be a strategy to reduce or eliminate replication-competent reservoirs.

Tae-Wook Chun (NIAID) described recent clinical studies testing therapeutic strategies to achieve a sustained viral remission in the absence of ART and advocated for the inclusion of ATI in studies to evaluate the impact of interventions aimed at controlling viral rebound. His data comparing HIV reservoirs and several immune parameters before, during and after treatment interruption showed no lasting effects or safety concerns related to the ATI [37]. Dr Chun also pointed out the necessity of including placebo control groups in clinical trials involving ATIs. In a recent NIH study testing a therapeutic vaccine, nearly 40% of the placebo-treated individuals maintained a reduced viral load after 4 months of treatment interruption [1]. The level and dynamics of viral control were varied among participants. Some maintained tight control of viral load; whereas, others experienced intermittent control, with a rapid rebound followed by extended periods of suppression without ART.

Jonathan Li (Brigham and Women's Hospital, USA) described his efforts to collect samples and clinical data from post-treatment controllers and non-controllers from multiple studies, including the NIH study described by Dr Chun, various other cohorts, and ACTG trials. Termed the CHAMP study [42], post-treatment controllers were defined as individuals with no evidence of pre-existing spontaneous HIV control before ART initiation who then discontinued

ART and maintained viral control, with viral load less than 400 copies/mL for at least two out of three time points measured over 24 weeks. The frequency of post-treatment control was greater in individuals who initiated ART during early infection (13%) compared to those who began treatment later during chronic infection (4%). Neither pre-ART viral load nor T cell activation levels predicted the ability to control virus, but single genome proviral amplification showed that post-treatment control was associated with a seven-fold smaller HIV reservoir before ART interruption (including both intact, replication-competent and defective proviral sequences). The duration of HIV remission was variable, with 50% of individuals remaining suppressed at 2 years after interruption, and 20% suppressed at 5 years, highlighting the need for regular viral load monitoring of post-treatment controllers. Some individuals showed initial viral rebound followed by suppression, suggesting the involvement of an effective antiviral immune response.

Discussion following this session focused on additional factors which potentially contribute to post-treatment control, including data from the VISCONTI cohort suggesting that participants with intermittent viraemia were more likely to rebound and restart ART, compared to participants who maintained an undetectable viral load.

Conclusion

This year's meeting highlighted several new advances that are likely to open up new avenues for HIV cure-related research over the next few years. Technological advances in single-cell analysis, *in situ* tissue staining, and whole-body imaging have the potential to help us better understand the nature of the persistent HIV reservoir and the effects of interventions in much greater detail than has so far been possible. The IPDA assay has the potential to become the new gold standard for measuring the size of the functional reservoir, with improvements in accuracy, precision, and/or throughput as compared to existing assays. Bar-coded viruses and phylogenetics can increase our understanding of the dynamics of the reservoir over time. Advances in overcoming latency and facilitating reservoir clearance *in vitro* or *in vivo* with TLR agonists, BCL-2 antagonists, and SMAC mimetics open new paths towards the ultimate goal of reservoir eradication and sterilising cure. The results of planned clinical trials of TLR agonists in combination with bNAbs, in particular, are greatly anticipated. Progress continues to be made on the engineering of optimised mAb-based therapeutics and CAR T cells to target reservoir cells, although identifying the best strategies for scale-up and testing in combination studies will continue to be a challenge for the field. The early success and safety of bNAb combinations in the clinic, or (in NHP) combinations of bNAbs with immune modulators such as a TLR7 agonist or anti-PD-L1 mAb, have helped to stoke enthusiasm for combined immunotherapies for long-term control of viral rebound. Screening participants for pre-existing resistance to bNAbs will be a critical requirement for future clinical trials testing these strategies. A greater appreciation for the frequency of spontaneous post-treatment control following early ART is providing encouragement for developing interventions to induce immune control in a broader population, but it also necessitates the inclusion of sizable placebo control arms in future clinical trials involving ATIs. The good news is that a consensus is building within both the research community and the participant community that ATIs are necessary to determine outcomes in trials of interventions aimed at achieving long-term immunological control of viraemia and, if designed properly and ethically, can be carried out safely, without measurable harm to the participant.

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Conflict of Interests

All authors declare no conflict of interest.

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