Highlights from the Third Biennial Strategies for an HIV Cure Meeting 14–16 November 2016, Bethesda, MD, USA

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Abstract

Since the first Strategies for an HIV Cure Meeting organised by the National Institute of Allergy and Infectious Diseases (NIAID) in 2012, one of the primary purposes of the meeting has been to facilitate communication and foster collaboration across the NIAID-funded Martin Delaney Collaboratories for HIV cure research (MDC), the broader HIV cure-related research field, and industry and community stakeholders. This year's meeting agenda reflected NIAID's increasing investment over the last 5 years in research to identify strategies for eradicating or achieving long-term remission of HIV infection. Overviews and research highlights were presented from each of the Martin Delaney Collaboratories, as well as projects funded through the Beyond HAART programme, the Consortia for Innovative AIDS Research in Nonhuman Primates (CIAR) programme, the ACTG and IMPAACT clinical trial networks, and the NIAID Vaccine Research Center in hopes of stimulating cross-talk and synergy among these and other programmes focused on HIV cure research. Aside from the oral presentations described here, the meeting also included 75 poster presentations. Finally, community engagement activities and community participation in the MDC was highlighted throughout the first day and in a special session on Day 2. This reflects NIAID's commitment to engage community partners in the earliest stages of research towards curative interventions through the MDC programme. The entire meeting is available for viewing via the NIH VideoCast website at: https://videocast.nih.gov/PastEvents.asp.

Day 1 keynote: addressing HIV persistence – a novel approach involving α4β7 integrin

Anthony Fauci, the Director of NIAID, began the meeting with a keynote presentation describing a surprising result from treating SIV-infected rhesus macaques with a primatised monoclonal antibody (mAb) against the α4β7 integrin that blocks trafficking of CD4+ T cells to the gut. In collaboration with Aftab Ansari, eight acutely infected monkeys (5 weeks post-infection with SIVmac239) were treated with antiretrovirals (ARVs) for 90 days in combination with a series of eight doses of an α4β7 monoclonal antibody [1]. All eight animals maintained either complete control or transient blips of viraemia for over 9 months after discontinuation of all treatment. The half-life of the antibody was 11.4 days. In contrast, the virus rebounded within 2 weeks in seven control animals receiving ARVs plus a non-specific negative control antibody. The mechanism by which the α4β7 antibody treatment led to this sustained viral remission is not yet clear. Several correlations were observed, however, including recovery of CD4+ T cell subsets, increases in cytokine-producing natural killer (NK) cells, antibodies to the gp120 V2 domain, biomarkers associated with reduced inflammation and recovery of retinoic acid levels. Clinical trials in HIV-positive individuals are now underway with vedolizumab, a humanised version of the α4β7 antibody currently approved for treatment of inflammatory bowel disease, to see if similar effects can be observed in humans.

Sessions 1 and 2: overviews of the Martin Delaney Collaboratories for HIV cure research

The Martin Delaney Collaboratories for HIV cure research (MDC) programme, originally begun in July 2011, 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 was designed to facilitate partnerships between academia, industry, government and community to move HIV cure research forward more rapidly than could be accomplished by individual groups working alone. In July 2016 the programme was expanded from the original three collaboratories to a total of six collaboratories, in part associated with President Obama's 2013 World AIDS Day proclamation to redirect $100 million in National Institutes of Health funding towards HIV cure research.

BELIEVE: bench-to-bed enhanced lymphocyte infusions to engineer viral eradication

Douglas Nixon provided an overview of BELIEVE's structure, investigators and major scientific objectives. Based at the George Washington University, BELIEVE is well positioned to address the high proportion of persons living with HIV in the greater Washington DC area. He introduced the community engagement leaders, Manya Magus and Amanda Castel, and the industry partnerships with Altor Bioscience and Torque. He briefly described the four initial research foci (IRF). IRF1: developing natural and engineered cytotoxic T lymphocytes (CTLs); IRF2: combining NK cells with broadly neutralising antibodies (bNAbs) to target antibody-dependent cell-mediated cytotoxicity (ADCC); IRF3: directing immune effectors to viral sanctuaries in lymphoid tissues; and IRF4: combining T cell and/or NK cell therapy with an IL-15 superagonist.

Brad Jones presented intriguing new data addressing the question whether CTLs can eliminate reservoir cells containing intact versus defective HIV provirus using the HIV eradication (HIVE) assay developed in his laboratory. Work in collaboration with Ya-Chi Ho demonstrated that although CTL kick and kill reduced overall HIV DNA, much of the reduction could be attributed to defective proviruses expressing HIV antigens. Surprisingly, CTL kick and kill failed to reduce the infectious viral reservoir. Dr Jones then introduced the IRF1 studies conducted by Mario Ostrowski and

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Thomas Smithgall to identify small molecule inhibitors of Nef to enhance CTL killing of latently infected cells. This IRF team will continue to explore different strategies in achieving elimination of intact reservoirs.

Elizabeth Connick described collaborations with Pamela Skinner and Gregory Burton in IRF2 to direct CTLs to B cell follicles within lymph nodes. HIV is concentrated in CD4+ T cells in B cell follicles. However, CD8+ CTLs tend to be excluded from B cell follicles and fail to accumulate at sites of virus replication in follicles [2]. Thus B cell follicles represent an important potential anatomical reservoir that will be the focus of IRF2. Their overarching strategy will be to target virus-specific CTLs to B cell follicles by transduction of the follicular homing chemokine receptor, CXCR5.

Manya Magnus discussed BELIEVE's goals towards meaningful community engagement. Based on prior research and stakeholder feedback, their community engagement guiding principles are structured around inclusivity, transparency, and breadth. She emphasised the importance of local engagement given the diverse BELIEVE research sites across Washington DC, Brazil, Canada, and Mexico. They will seek community guidance through all phases of projects, including protocol and consent development, outreach, recruitment, retention and information dissemination. She closed by describing their comprehensive process to continually evaluate and improve engagement strategies.

DARE: Delaney AIDS research enterprise to cure HIV

Steven Deeks discussed research plans for the DARE Collaboratory, based at the Univeristy of California, San Francisco, with goals centred around enhanced killing of infected cells and durable immune-mediated control of HIV, particularly in lymphoid tissue reservoirs. As previous efforts to activate and eliminate the residual viral reservoir have failed, and any residual functional virus can cause clinical consequences after removal of antiretroviral therapy, the team aims to generate a sustained and efficient cell-mediated killing response that will be primed and activated to specifically target any cell that begins producing virus. The research is focused on the need for a thorough understanding of the mechanisms of virus persistence, directed therapies to target the infected cells, and biomarkers that directly and accurately quantify HIV in tissue reservoirs.

Louis Picker presented evidence that B cell follicles in lymphoid tissues are sites of residual HIV infection that are protected from effective cellular antiviral immune responses. Planned non-human primate (NHP) studies will test whether maintenance of the T follicular helper cell reservoir is due to limited antiviral cellular responses or intrinsic properties of this cell population. Therapeutic approaches involving combinations of follicle disruption, therapeutic vaccination [3], latency reactivation and/or immune checkpoint blockade will be tested in NHP to determine whether these lead to control or elimination of the viral reservoir.

Timothy Henrich described plans to use highly sensitive whole-body positron emission tomography (PET) imaging as an innovative approach to better identify and quantify HIV reservoirs in lymphoid tissue and elsewhere in the body, including the brain. Intravital PET imaging would help to overcome the sampling error, limitations in cell number and discomfort to the study subject that is associated with tissue biopsies.

Sharon Lewin described efforts to reverse T cell exhaustion, reactivate latent virus, and enhance SIV/HIV control by inhibiting PD1, CTLA-4, and other immune checkpoint modulators. Similar strategies have shown great promise in treating cancer, and many licenced antibodies and other agents are available to test in humans. However, there are biological differences and potential issues with side effects that warrant caution in proceeding with clinical studies.

defeatHIV: Delaney cell and genome engineering initiative

Keith Jerome began the overview of the defeatHIV collaboratory, based at the Fred Hutchinson Cancer Research Center, with their unifying hypothesis that cell and gene therapies represent promising approaches to a sustained viral remission or sterilising HIV cure. Accordingly, their overarching goal is to first identify cell and gene therapy strategies assessable in NHP that can then be tested in small-scale human trials. He introduced their three IRFs. IRF1: chimeric antigen receptor (CAR) T cell therapy in combination with a latency-reversing agent (LRA); IRF2: delivery of the synthetic HIV-neutralising molecule, eCD4-Ig; and IRF3: gene-protected T cells in combination with therapeutic vaccination. He described the experimental schematic to co-ordinate the different IRFs and their respective non-human primate studies.

Hans-Peter Kiem summarised progress and lessons learned from the first iteration of defeatHIV. This early work successfully demonstrated the feasibility of zinc finger nuclease (ZFN)-mediated CCR5 gene disrupted stem cell engraftment in pigtail macaques [4], which they now plan to combine with CAR T cell approaches to target reservoir cells. He described their experimental approach to modelling CAR T cell therapies in macaques. In IRF1, the team will develop HIV-resistant anti-HIV CAR T cells. He showed preliminary data from Thor Wagner and David Rawlings demonstrating efficient cellular expansion and CCR5 gene disruption in macaque T cells treated with their anti-HIV CARs. In IRF3, James Mullins and Deborah Fuller aim to develop approaches for a therapeutic vaccine targeting conserved elements of the viral proteome. Altogether, these IRFs will focus on immune and vaccine strategies using HIV-protected T cells.

Larry Corey followed with further discussion of anti-HIV CAR T cell therapy approaches. Their aims are to first optimise production and cellular phenotypes of genetically protected anti-HIV CAR T cells in animal models, and then move to examine the ability of protected CAR T cells to kill human HIV reservoir cells *ex vivo*. Studies will include evaluation of engraftment, trafficking to sanctuary sites, proliferation and persistence of these protected anti-HIV CAR T cells *in vivo*. Importantly, he noted these approaches will require careful monitoring for possible cytokine release syndrome or neural toxicity effects.

Michael Louella presented the defeatHIV perspectives on meaningful community engagement. He emphasised that engagement requires the creation of relationships to align interests and foster co-operation. Their community advisory board (CAB) has a robust set of ongoing community engagement strategies including a variety of meetings and events, community conversations, webinars and social media. Their mission statement is to serve as a communication link and mobilise researchers and the community to work together to cure HIV infection. He closed the presentation by highlighting integrated interactions both within and beyond defeatHIV.

BEAT-HIV: Delaney collaboratory to cure HIV-1 infection by combination immunotherapy

Luis Montaner began the afternoon session with an overview of the BEAT-HIV collaboratory, based at the Wistar Institute, which will focus on generating cellular and innate immune responses to achieve long-term control and elimination of HIV-infected cells. Current efforts involve clinical studies designed to optimise and

combine strategies for type I interferon (IFN)-induced antiviral responses [5] and broadly neutralising antibodies to stimulate NK cell elimination of persistently infected cells. In addition, the team will develop cell-based therapy for long-term HIV control using HIV-specific engineered CAR T cells that attack infected cells and are resistant to infection. The impact of these treatment strategies on latent and active virus-producing HIV reservoirs in tissue and stem cell compartments will be explored.

Robert Siliciano presented detailed phylogenetic analyses showing that intact proviruses that are non-inducible in viral outgrowth assays can still be replication-competent when cloned and expressed, suggesting that quantitation of intact proviral sequences provides a more accurate quantification of the entire replication-competent reservoir compared to standard DNA PCR assays and even quantitative viral outgrowth assays (QVOA) [6]. Additional studies provided evidence that the majority of the latent reservoir is actually derived from clonal expansion of cells containing replication-competent provirus – a much larger proportion than originally thought. As most known drivers of T cell proliferation are expected to reverse latency, the team will further explore mechanisms that maintain latent reservoirs during proliferation.

Marina Caskey described animal and clinical findings demonstrating reduced viraemia following broadly neutralising antibody (bNAb) administration, and delayed rebound following antiretroviral treatment interruption [7]. Viral escape variants were observed but these viruses were sensitive to other bNAbs, so the group plans to evaluate treatment with combinations of bNAbs in viraemic individuals to determine effects on the latent reservoir size and on viral rebound following antiretroviral treatment interruption.

James Riley described strategies to improve CAR T cell specificity and function to optimally control HIV replication in the absence of ART, and to protect those effector cells from infection through either ZFN-mediated modification of the CCR5 gene [8] or engineered expression of a cell surface-expressed C34-CXCR4 fusion inhibitor [9]. Planned clinical studies will explore whether engineered T cell therapy can reduce the viral reservoir in infected individuals, what proportion of modified cells are needed to reduce the reservoir, and whether long term viral control can be achieved with CAR T cells alone or in combination with other immunomodulatory strategies.

CARE: collaboratory of AIDS researchers for eradication

David Margolis presented the current goals of the CARE collaboratory, based at the University of North Carolina, Chapel Hill. The mission of this collaboratory is to develop and validate combined strategies to reactivate latent HIV and recruit an effective anti-HIV immune response to clear infected cells using engineered antibodies, expanded antigen-specific T cells, and/or therapeutic vaccines. Additional goals include developing improved assays to detect clonal expansion of latently infected cells, viral antigen expression and molecular signatures of latently infected cells.

Daria Hazuda, from industry partner Merck Research Laboratories, presented an in-depth view of the approach for discovery of novel LRAs, including assays for ultrasensitive detection and quantitation of HIV antigen expression post-reactivation of latent HIV, evaluation of the mechanisms of action of novel LRAs to enable optimisation of potency without undesirable off-target effects. Dr Hazuda also discussed the determination of combinations of LRAs that can produce synergistic effects for improved efficacy and reduced toxicity.

Guido Ferrari described efforts to generate and optimise bNAb-based dual affinity retargeting (DART) molecules, engineered to bring cells expressing HIV antigen together with cells primed to recognise and kill those cells [10]. The goal of these studies is to promote killing of infected cells after administration of an LRA; current studies are aimed at determing the level of HIV antigen expression required for effective killing and which antibody moieties will be most effective when engineered into DART molecules.

Ann Chahroudi summarised ongoing related clinical studies using vorinostat for HIV reactivation combined with a dendritic cell-based therapeutic vaccine or expanded antigen-specific T cells (HXTC). In addition, detailed plans for sustained treatment interruption studies in NHP and mouse models were described, which will test a variety of novel and risky combination interventions, evaluate cellular and tissue reservoirs over time, and optimise multiple variables in ways that are difficult to achieve in clinical studies.

I4C: combined immunological approaches to cure HIV-1

The sixth and final MDC overview presentation was given by Dan Barouch who introduced the overall strategy of the I4C collaboratory, based at the Beth Israel Deaconess Medical Center (BIDMC), which is to rapidly eliminate virally infected cells using combinations of bNAbs and LRAs, and combine this approach with therapeutic vaccines aimed to boost the host immune responses for long-term control of virally infected cells.

Dr Barouch presented preclinical data consistent with bNAb therapy (PGT121) or a therapeutic vaccine (Ad26/MVA) in combination with a Toll-like receptor (TLR) 7 agonist having a partial impact on the size of the viral reservoir and time to viral rebound [11, 12]. In their IRF1, individual vaccine approaches will be tested in humans that initiated treatment during acute infection. Janssen will provide the Ad26/MVA therapeutic vaccine for a small pilot clinical trial. Their IRF2 aims to inform next generation cure strategies by defining mechanisms for control of the reservoir in NHPs and optimisation of bNAbs. Industry partner, Gilead, will provide TLR7 agonist and ARVs for the NHP studies.

Nelson Michael provided further details of the Ad26/MVA therapeutic vaccine study where 36 rhesus macaques were started on ARVs 7 days after infection [12]. At 24 weeks post-infection, animals received either placebo treatment, Ad26/MVA vaccine alone, TLR7 agonist GS-986 alone, or the combination of Ad26/MVA with TLR7 agonist. At 72 weeks post-infection ARVs were discontinued. Animals that received the TLR7 agonist had increased activation of CD8 T cells, and animals that received the Ad26/MVA vaccination had a 9.2-fold expansion in cellular immune breadth. The group receiving the combination of Ad26/MVA plus TLR7 agonist had a 2-log reduction in the mean viral set point as compared to placebo or TLR7 agonist alone. While all animals eventually rebounded following ARV interruption, the combination group had an average viral rebound at 21 days compared to an average rebound at 10–15 days for the other groups.

John Mellors advocated that 'proof of concept' *in vivo* therapeutic responses are needed to drive the cure field forward. The I4C Collaboratory focuses on therapeutic vaccines using conserved viral antigens for maximum breadth of immune response and reduced risk of immune escape. The initial clinical studies will focus on individuals treated with ARVs at the earliest stages of infection, with the rationale that there should be fewer infected cells, less viral diversity and a relatively intact immune system that can be manipulated to direct responses towards the virus. Planned clinical trials include a small pilot trial of Ad26/MVA mosaic antigen vaccine provided by industry partner, Janssen, in individuals treated during acute infection in South Africa and a small pilot trial of a pulsed peptide dendritic cell vaccine [13] in acutely treated

individuals in Thailand. Community members from Boston, Pittsburgh, South Africa and Thailand will advise the I4C on the planned pilot clinical trials.

Galit Alter presented data suggesting that humoral biomarkers are predictive of viral rebound. In collaboration with Thomas Rasmussen and Ole Sogaard, human antibodies were interrogated before and after *in vivo* administration of the LRA, panobinostat [14]. The data showed a strong inverse correlation between antibodies that recruit neutrophils and/or complement and the size of the HIV DNA reservoir. Likewise, in the Ad26/MVA plus TLR7 agonist combination study presented by Dr Michael, systems serology analysis identified an increase in antibodies that activate NK cells in those receiving the combination [15]. NK cell activity was significantly associated with time to viral rebound and correlated inversely with viral set point.

Day 2 keynote: clinical trials in HIV cure – where have we been, where should we be going?

Daniel Kuritzkes opened the second day with a keynote presentation providing an overview of prior clinical studies for HIV cure. Although stem cell transplantations can substantially reduce HIV reservoirs, some infected cells apparently persist in anatomical reservoirs that are inaccessible to typical clinical sampling and cause virological rebound upon treatment interruption (TI). Gene therapy can be used to safely engineer HIV-resistant cells that persist after infusion and become enriched during TI. However, curing HIV will be likely to require combining gene therapy strategies with other strategies that eliminate latently infected cells. Enthusiasm for LRAs was dampened recently by the discovery that potent LRAs fail to reactivate all proviruses *ex vivo*, suggesting that it may be challenging to safely reactivate all proviruses *in vivo*. Enthusiasm for immune checkpoint inhibitors and other immunomodulators remains high but is also tempered by safety concerns and the uncertain risk/benefit ratio for healthy HIV-infected persons currently on ARVs. Therapeutic vaccines have demonstrated immunogenicity in humans but have failed thus far to replicate the promising virological results observed in NHPs, suggesting a need for more systematic human studies aimed at defining relevant end points and correlates of efficacy. Infusion of individual bNAbs has only modestly delayed time to rebound thus far, suggesting that combinations of bNAbs may be required to maintain viral suppression and prevent the emergence of resistant variants. Finally, recent data indicate that very early initiation of ARVs appears to limit, but not prevent, reservoir seeding. In closing, he encouraged rigorous inclusion of control groups during exploratory cure trials to ensure that promising, but low frequency, responses reflect true impacts of the interventions being studied.

Session 3: clinical studies, paediatrics, and early treatment

Rajesh Gandhi discussed ongoing cure studies in the AIDS Clinical Trials Group (ACTG). One particularly valuable resource for cure researchers could be the ACTG's well-characterised cohort of infected persons on ARVs who provide blood samples twice a year; a subset also contributes tissue samples. Recent analyses of this cohort have established that the best correlates of on-ARV HIV persistence, inflammation and T cell activation are not other on-ARV measures, but rather pre-ARV measures. This finding suggests that the 'die is cast' before ARV initiation, and interventions targeting inflammation or activation may fail to impact HIV persistence. Joseph Eron then explained how other researchers can work with the ACTG by requesting data to perform new analyses, collaborating on studies of existing samples, and proposing new interventional trials.

Deborah Persaud reviewed cure research efforts in the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) network. Prior IMPAACT studies have helped to expand treatment options for infants. Ongoing studies aim to expand treatment options for paediatric populations, reduce reservoirs with very early ART treatment and interventions like the VRC01 bNAb, and evaluate impacts of CCR5-delta32 cord blood transplants for children who require transplantation for underlying disease. Studies are underway to understand the CNS reservoirs and the relationship between viral reservoirs and the developing immune system in children. The overall goal is to identify safe, feasible and scaleable HIV eradication strategies for paediatric populations.

Jintanat Ananworanich demonstrated that early initiation of ARVs (i.e. during Fiebig stage III/IV) significantly delays the time to rebound during TI and also reduces the rate of viral load rise when compared with initiation of ARVs during chronic infection. However, the observed 8-day delay in time to rebound was modest, unlikely to be clinically meaningful, and suggests that durable remission may require immunotherapy in addition to early treatment. Professor Ananworanich also called attention to the recent study of anti-$\alpha 4\beta 7$ mAb in NHP, where substantial rises in viral load preceded viral control. She noted these results raise concern that clinical trials for HIV cure may miss beneficial outcomes if ARVs are resumed as soon as HIV plasma RNA rises above 1000 copies/mL, as is the current practice in many TI studies. The discussion following this session revealed a lack of consensus with regard to whether trial participants should be allowed to remain off ARVs with higher viral loads for extended periods of time. It was noted, for example, that data are lacking as to whether prolonged periods off ARVs can re-seed replication-competent viral reservoirs, which remain a challenge to measure quantitatively.

Session 4: community engagement, social science, and ethical considerations for HIV cure research

Judy Auerbach described how little social science research has been done to date [16]. Questions of interest include exploring stakeholder perspectives; exploring how different communities understand the concept of cure; analysing how life histories and identities of people living with HIV are affected by cure research; exploring sex and gender differences; and delving into issues of equity, access and cost in research and implementation. Stakeholders of interest include people living with HIV (PLWH), clinicians, researchers, policy makers, funders and the general public. PLWH may face risks in cure research that could compromise their own health while contributing to the research enterprise, and clinician-researchers may also struggle with allowing risks to HIV-positive patients. Stigma is still a cross-cutting issue, and can be both a deterrent and a motivator for research participation. At the policy level, funders and policy makers are interested in whether cure strategies will be cost effective or cost-saving, and will be concerned with questions of equity and access. Stakeholder engagement strategies used in HIV prevention trials provide important tools and lessons learned that can be applied in HIV cure research [17]. Various understandings of the concept of cure have been explored in studies in China [18] and South Africa, revealing nuanced meanings in different cultures and languages. In South Africa, experience with spurious claims of 'cures' based on herbal medicine have complicated discussions of cure research. Further studies are needed to understand the cultural and linguistic implications of the concept of cure. Early HIV cure research studies suggest possible sex differences in certain biological responses, highlighting the importance of inclusion

of women. Gender differences may play a role in willingness to participate, as women's caregiving roles may lead to greater difficulty in joining a trial. Questions of access and representativeness are also relevant for different disease states. Currently, most cure studies only enroll participants with undetectable viral load, although those whose viral load is not well-controlled have the most need of new interventions.

Regan Hoffmann, a policy expert, advocate, and person living with HIV for more than 20 years, shared her personal experience of living with the disease. She commented that stigma follows a person everywhere and that HIV infection is a constant reminder of one's mortality. Even while accessing high-quality medical care and treatment, fear is ever-present. New policies and laws relating to criminalisation of HIV transmission have also contributed to fear in the community. Ms Hoffmann described how the promise of a future cure intervention can give hope and inspiration to those battling the disease, and will be essential to a final end to the epidemic worldwide.

Nir Eyal addressed ethical challenges of TI in studies investigating viral control after a period of transient high viral rebound as highlighted by Professor Ananworanich in Session 3. He noted that the studies involve risks not only to the study participants, but also risks of transmission of HIV to third parties. Risks to study participants can be mitigated or managed with a variety of techniques including careful selection of participants, close monitoring and clear guidelines for restarting therapy, among other measures. Managing risks to third parties may include these and additional procedures such as counseling on safe sex, offering PrEP to partners of study participants or seeking informed consent from partners. One concern raised anecdotally was clinical trial sponsor reluctance to assume responsibility for third-party risks. There is no legally binding obligation to take on responsibility for third-party risks. Voluntary measures, therefore, should not be seen as assumption of full liability for HIV exposure risk. A final point was that risks to third parties cannot be completely eliminated in TI studies and that these studies, therefore, pose novel challenges for research ethics, which usually concerns itself almost exclusively with obligations towards the welfare of enrolled subjects. TI studies may engender a special responsibility on the part of researchers to consider these third-party effects, and further work on these research ethics questions is needed.

Allison Matthews concluded the session with a presentation on crowdsourcing as a novel method of improving community engagement. Traditionally HIV research relies on community advisory boards (CABs) for engagement but, despite their value, there are limitations to what CABs can accomplish. Crowdsourcing can be used to generate interest, discussion and problem solving from a grassroots level. One example of a crowdsourcing project consisted of a contest for members of the public to respond to the question, 'What does HIV cure mean to you?' via submission of art, video and music entries. The crowdsourcing exercise revealed important insights: some community members expressed that they were afraid to contribute because they felt uninformed, and many said that HIV was only one of many vulnerabilities faced by their communities. A number of myths and misconceptions about HIV cure were revealed and discussed, including the idea that a cure already exists and is being withheld from the community or that a holistic diet and healthy living can cure the disease. A second crowdsourcing contest addressed communication strategies, and contest winners proposed a new app to be developed specifically for HIV-related communication. In summary, the crowdsourcing strategies provided innovative means to engage with diverse individuals for problem solving that is responsive to community perspectives and needs.

Session 5: HIV cure-related basic research

Stephen Hughes opened the session with evidence that ongoing viral replication does not explain the persistence of HIV in most patients. He then focused on clonally expanded cells that harbour HIV DNA, acknowledging that many of these integrated proviruses are defective and cannot make infectious provirus. In one case, however, a single infectious proviral clone, Ambi-1, was recovered from an individual (Patient 1) and this clone was the source of viraemia for years [19, 20]. In another study, six patients who started treatment approximately 3–14 weeks after infection were evaluated. At the time treatment was initiated, expansion of clones with unique HIV integration sites was detected in three of the six patients. For all six patients, expanded clones were detected at 2–3.5 years after treatment initiation. For two of these patients the same clone was detected at the time of treatment initiation and years later. These data are consistent with the establishment of HIV clones early in infection that persist for years while the patient is on therapy.

Mary Kearney presented a model using simulated viral sequences that assumed a baby was infected perinatally and then was suppressed on ARVs for 8 years. Using the substitution rate published in Lorenzo-Redondo *et al.* [21], the model predicted that if active ongoing replication were taking place within lymph nodes for 8 years despite ARV treatment, the sequences would be divergent from those taken at early time points. However, real-life single genome amplification data from three children with perinatal HIV infection who were treated for 4–7 years demonstrated that for each case the sequences isolated after years on ARVs did not diverge from pre-ARV viral sequences. This was the case for virus isolated from both the blood and lymph nodes. These data support the conclusion that ongoing viral replication is not occuring in these individuals. In a separate study, analysis of samples taken from Patient 1 described by Malderelli *et al.*, and referred to by Dr Hughes above, demonstrated that only 3% of cells in an expanded clonal population of HIV expressed RNA [19]. This most likely explains how clonally expanded cells containing replication-competent HIV provirus are able to persist without being recognised by the immune system.

Eli Boritz described a study where subsets of CD4+ T cells from the blood and lymphoid tissue of HIV controllers were sorted by flow cytometry, and single copies of viral sequences were obtained from each subset to gain insight into HIV persistence in the presence of effective antiviral immunity. Most of the HIV-infected CD4+ T cells in the blood came from a few precursors that were infected in the distant past and underwent massive clonal expansion. His data were consistent with those from Drs Kearney and Hughes, that only a small subset of the clonally expanded cells are transcriptionally active. In lymphoid tissue from HIV controllers, most cells, and in particular the T follicular helper subset, are recently infected and transcriptionally active, and a small subset of the total infected cells link the lymphoid reservoir to the blood [22].

Eirini Moysi gave an overview of the defined cellular architecture of lymph nodes of uninfected individuals and how HIV infection alters this environment. Dr Moysi presented data for HIV-positive individuals on treatment who received the influenza vaccine. At pre-vaccination for influenza, HIV-positive individuals on ARVs had a higher baseline of T follicular helper (Tfh) cells compared to HIV-individuals. The higher baseline frequency of Tfh cells in HIV-positive individuals corresponded to a higher antibody titre directed towards influenza, even though there was a loss of Tfh cells due to death of cells in the germinal centre. The drop in the Tfh cells correlated with a drop in the HIV plasma viral load and lower levels of HIV early transcripts in lymph node cells.

Ya-Chi Ho reminded the audience that the majority of HIV-1 proviruses in patients on ARVs are defective due to APOBEC-mediated G-to-A hypermutations, large internal deletions, and packaging signal/major splice donor deletions and mutations [23]. The frequency of clonally expanded HIV-infected T cells increases over time in patients suppressed on ARVs. Defective proviruses can be transcribed and translated *in vitro* and transcribed *ex vivo* [6] , and CTLs appear to be required to maintain viral suppression in macaques on ARVs [24]. Recent data from a collaboration with Brad Jones from the BELIEVE collaboratory demonstrate that cells containing most types of defective HIV proviruses can be recognised by CTLs *in vitro*. Surprisingly, the defective proviruses seem to have a diluting effect on the CTL response to cells that harbour full-length proviral DNA and express HIV protein. These data are consistent with a model in which cells expressing defective proviruses act as decoys for the CTLs, thus preserving the replication-competent latent reservoir.

Fabio Romerio gave an overview for how nucleosome assembly at the 5′ LTR is important for HIV latency and EZH2 is the dominant histone methyltransferase that acts at the 5′LTR [25]. EZH2 is a component of the larger polycomb repressive complex 2 (PRC2) that is recruited to promotors by long non-coding RNA (lncRNA). The antisense transcript of HIV-1 has been reported to act as a lncRNA [26,27]. Dr Romerio used a single-strand RT-PCR assay to detect the HIV-1 antisense (Ast) RNA from resting T cells isolated from patients suppressed on ARVs. To interrogate the role Ast might play in latency, Dr Romerio used the Karn Jurkat cell model of latency to show that cells overexpressing Ast RNA exhibit delayed reactivation from latency. Conversely, when latent cells were first stimulated with LRAs, cells overexpressing Ast RNA re-established latency more quickly as compared to those not expressing Ast. RNA Pol II ChIP assays revealed that cells overexpressing Ast did not have RNA Pol II at the promotor, indicating no transcription. ChIP assays detecting methyltransferase EZH2, PRC2, and methylation are consistent with Ast acting to silence HIV-1 at the promotor. This is consistent with the recent confirmation of the long-standing hypothesis that Ast plays an important evolutionary role as the tenth gene of HIV [26,28].

Session 6: preclinical HIV cure research

This session provided overviews of the recently funded NIAID Consortia for Innovative AIDS Research in Nonhuman Primates (CIAR) and The Foundation for AIDS Research (amfAR) Institute for HIV Cure Research.

Dan Barouch gave an overview of the Harvard CIAR, focusing on two distinct HIV-1 vaccine candidates: (1) Ad26-SIVsmE543 *gag/pol/env* prime with SIVmac32H Env boost (Ad/Env), and (2) rhesus CMV (RhCMV) expressing SIVmac239 genes (CMV/SIV). The Harvard CIAR has two foci: Focus 1 evaluates prophylactic HIV vaccines, and Focus 2 evaluates HIV/SIV eradication strategies. Focus 1 investigates the mechanisms of protection by the Ad/Env and CMV/SIV vaccines both individually and in combination. Focus 2 evaluates therapeutic vaccination, engineered autologous CD8 T cells, and novel vectors, inserts and regimens as SIV eradication strategies. A 90% reduction of per-exposure acquisition risk and 50% complete protection was seen when the Ad26-Env vaccine was given prophylactically [29]. The Env protein boost increased the production of antibodies with Fc functionality, which correlated with enhanced protective efficacy. Sixty-six percent protection was achieved with the Ad26/Ad26+Env vaccine against heterologous SHIV-SF163P3 challenge. The high-level clinical development plan for the Ad26/MVA HIV vaccines, which have moved from Phase I to Phase II trials, was also presented.

Louis Picker focused the second part of the Harvard CIAR talk on understanding the mechanism of action of CMV/SIV vaccines. The overall efficacy of RhCMV vectors in clearing infection is 55% (against SIVmac239) when given prophylactically [3]. The unique protection pattern involved an 'effector memory-type' control and clear response, and was binary (complete or none at all). The critical questions for vector development are: (1) What cell type/mechanism determines efficacy?; (2) What determines viral clearance versus lack of clearance?; and (3) Can the efficacy be increased by optimising vector design? The investigators found that CMV vectors can be programmed to elicit CD8+ T cells with distinct epitope targeting patterns, including both conventional MHC-1a-restricted epitopes and unconventional epitopes restricted by MHC-II and MHC-E. In the future, the mechanism of action will be evaluated by immune depletion studies as follows: anti-CD8α antibody will be infused to deplete CD8+ T and NK cells, anti-CD8β antibody will be used to deplete CD8αβ+ T cells, anti-IL-15 antibody will be used to block NK and partially T effector memory cells and anti-CD20 antibody will be used to destroy B follicle sanctuaries. Downstream Harvard CIAR priorities are to determine whether antibody and cellular immunity strategies will synergise for prophylactic studies and to exploit similar vaccine strategies for curing infection in animals that were infected prior to vaccination.

Eric Hunter and Rama Amara gave a joint presentation on the Emory CIAR. Like the Harvard CIAR, Focus 1 studies mechanisms of prophylactic vaccine protection and Focus 2 investigates mechanisms for reservoir eradication. Focus 1 has three projects: project 1 investigates SOSIP gp140 trimers with novel TLR7 adjuvants (3M-052); project 2 studies tissue resident memory CD8 T cells; and project 3 explores strategies to balance humoral and cellular immunity. Focus 2, led by Guido Silvestri, examines immunotherapy for a functional cure. The major areas of study within Focus 1 are: testing of new adjuvants; studying T follicular helper cells and neonatal imprinting; the synergy between antibodies and CD8 responses; the modulation of susceptibility; and imaging and systems biology analyses. Focus 2 explores the use of anti-PD-1, anti-CTLA4, and LAG3 antibodies, and therapeutic uses of IL-10, IL21 and JAK inhibitors. In addition, the investigators plan to test CD8 depletion strategies and latency-reversing strategies combined with DART bispecific antibodies. Another goal is therapeutic vaccination using DNA/MVA delivery of SIV genes or heterologous viral vector vaccines (VSV prime, followed by a sequential vaccinia and Ad5 boost).

Paul Volberding presented an overview of the newly established amfAR Institute for HIV Cure Research based at the University of California, San Francisco. Their mission is to harness innate and adaptive immune responses to reverse latency and reduce and control the remaining HIV reservoir. TLR agonist studies focus on TLR4, TLR7, TLR8 and TLR9 (MGN-1703), and acitretin, which activates RIG-I sensing. The residual HIV reservoir is measured using RNA/DNA scope combined with multicolour staining of tissue using DAPI, αCD20 antibody and αCD3 antibody. In addition, FDG-PET/CT imaging of lymph nodes will be explored as a means to measure the HIV reservoir in humans. The consortium also includes assay development using, for example, Hologic ultrasensitive assay technologies and digital droplet PCR assays with Raindance Technologies. In addition, the amfAR Institute supports humanised mice and NHP studies testing TLR7 agonist (imiquimod) alone or in combination with TLR4 agonist (GLA). They also test the safety and efficacy of GS-9620, a proprietary TLR7 agonist from Gilead.

Day 3 keynote: developing T cell therapies for HIV – lessons from virus-specific T cell therapies post-transplant

Catherine Bollard opened the third and final day of the meeting by highlighting her laboratory's interdisciplinary expertise in virology and haematology. She began with an overview of the diversity of adoptive therapy T cell products, the rationales for using haematopoietic stem cell transplantation (HSCT) therapies, and initial work towards developing virus-specific T cell therapies post-HSCT. The early proof-of-principle studies and manufacturing processes for generating multivirus-specific T cells (VSTs) were cumbersome, and thus newer technologies have been developed to produce these cells in a shorter time. Using cord blood-derived dendritic cells, optimised cytokine cocktails, and antigen-presenting cells, they have been successful in generating a variety of VSTs. The VSTs were characterised for safety, efficacy and persistence. VST safety was assessed using alternative mismatched donor–recipient pairs. There was minimal toxicity and patients did not have clinically significant graft versus host disease (GVHD). Virus-specific CTL protection against cytomegalovirus (CMV) demonstrated high efficacy at a 93% response rate [30]. Comparable efficacy was also demonstrated with adenovirus and Epstein–Barr virus (EBV). VST persistence was assayed by TCR deep sequencing. This analysis demonstrated that clones harboured the same adenovirus-specific TCR clonotypes from the patients. The duration of T cell persistence depended on viral reactivation. Altogether, these data showed multivirus-specific T cells can be generated from two donor sources – peripheral blood and cord blood – and were safe with minimal toxicity. These VSTs persisted in the presence of antigen and were protective. VST therapies may also be developed in combination with other strategies, such as CAR T-cells. These studies set the stage for future applications for other virus-associated cancers. The most recent VSTs can target six different viruses (CMV, EBV, adenovirus, human herpes virus 6, BK virus and parainfluenza).

Moving on to HIV, Dr Bollard described a method for expanding HIV-specific T cells from HIV-positive individuals *ex vivo* by growing CD8 and CD4 T cells in the presence of antiretroviral drugs. Cells were exposed to a proprietary mixture of Gag, Pol, and Nef peptides to achieve broad coverage across all HIV clades. After 24 days, the resulting cells, referred to as HXTCs, exhibited HIV antigen specificity, produced polyfunctional responses, suppressed HIV *in vitro* and killed latently infected cells isolated from individuals after *ex vivo* treatment with the LRA, vorinostat [31]. Future directions will examine the re-infusion of HXTC cells back into individuals in combination with vorinostat treatment *in vivo*. Another study will combine broadly HIV-specific T-cells capable of targeting non-escaped epitopes with IL-15 superagonist as an LRA. Finally, Dr Bollard posed a provocative hypothesis that transplantation itself could potentially serve as an LRA in combination with HIV-seronegative CCR5Δ32 donor-derived HXTCs.

Session 7: Beyond HAART – innovative approaches to cure HIV-1

NIAID began the Beyond HAART programme in July 2012 with co-funding from the NIMH. Two projects were funded in the first iteration. The programme was then expanded significantly in 2015 with an additional large investment from the National Heart Lung and Blood Institute (NHLBI). The programme encourages research on non-drug-based approaches to control or eliminate HIV.

Ronald Desrosiers opened the session by presenting work completed in collaboration with Michael Farzan on the long-term delivery of bNAbs in NHP. Treatment with a combination of bNAbs delivered by adeno-associated virus (AAV) resulted in a reduction in the viral load to below the level of detection in one out of four SHIV-infected NHP. Ongoing studies attempting to recover virus from that one animal, adoptive transfer of lymph node cells to naive recipient animals, and additional animal challenge studies will help confirm the effects of the bNAb treatment on the viral reservoir. The efficacy of AAV-mediated delivery of bNAbs seemed to be negatively impacted by host anti-antibody responses, and efforts are being expended on modifying vector design and varying the route of administration to minimise anti-antibody responses and maximise delivery.

Kevin Morris presented transcriptional gene silencing studies using small interfering RNA (siRNA) directed against a region in the HIV LTR that may be involved in the establishment of viral latency. Other groups had shown that HIV expresses an endogenous antisense non-coding RNA that appears to regulate viral transcription. An siRNA targeting the LTR site that encodes for the lncRNA, delivered into infected cells using a gp120 aptamer, resulted in repression of HIV transcription and decreased viral expression in infected T cells [27]. Preliminary *in vivo* experiments in a humanised mouse model of latency also revealed that treatment with gp120 aptamer-siRNA reduced viral load and maintained CD4 cells upon ARV removal. These studies are one of the first examples of transcriptional gene silencing using exogenously delivered cell-targeted aptamer-siRNAs.

The four Beyond HAART programme projects awarded in 2015 are specifically focused on new approaches and research to understand, simplify and improve upon existing cell- and gene-based strategies to eliminate HIV-1.

David Scadden, in collaboration with CRISPR Therapeutics, is using CRISPR technology to genetically engineer haematopoietic stem progenitor cells (HSPCs) to be HIV-resistant, and CD8 T cells to have enhanced ability to destroy viral reservoirs. CRISPR-CCR5 edited HSPCs are able to engraft long-term with multi-lineage haematopoietic reconstitution in humanised mice and preferential selection upon HIV infection. A non-toxic conditioning regimen consisting of CD45 antibody conjugated to saporin, a protein synthesis inhibitor, is being explored to improve the engraftment of modified cells while minimising genotoxic effects.

Irvin Chen, in partnership with Calimmune, is pursuing an anti-HIV strategy using HSPC transplantation of genetically modified cells to be resistant to HIV infection in combination with CAR T-cells. Transplantation of pigtail macaques with HSPCs transduced with a lentiviral vector to reduce CCR5 levels and to express a membrane-bound fusion-inhibitor (C46 peptide) resulted in lower SHIV plasma viraemia and higher CD4 cells upon SHIV challenge compared to non-transplanted animals. Co-expression of a CD4-zeta CAR construct with C46 in HSPCs resulted in engraftment and differentiation of HSPCs into multiple haematopoietic lineages after transplantation in macaques, and expansion of cells in response to SHIV infection. Future plans are to enhance engraftment of gene-modified HSPCs and improve the CAR constructs with the long-term goal of a Phase I clinical trial using this 'defend and attack' approach.

James Riley, working with industry partner Sangamo Biosciences, is testing the protective effects of a cell surface-expressed chemokine receptor-associated fusion inhibitor (C34-CXCR4) in a clinical trial, while developing the next generation of cell-based, genetic approaches to control or cure HIV-1 infection. Preclinical studies showed that T cells transduced with C34-CXCR4 were resistant to infection from multiple strains of HIV and were preferentially protected from HIV infection *in vivo* in HIV-infected humanised mice. A dose-escalation Phase I clinical trial in

HIV-infected individuals is anticipated to start enrolment in 2017 and will assess the safety of C34-CXCR4-transduced T cells, the trafficking and immunogenicity of the engineered T cells, and potential effects on viral load, viral evolution and resistance. Additional studies are being conducted on targeted insertion of C34 into the endogenous CCR5 or CXCR4 gene to generate HIV resistant cells, optimisation of a CD4-CAR construct with improved anti-HIV activity, and characterisation of the transcriptional gene signature profile of T cell exhaustion in HIV infection.

Paula Cannon and Hans-Peter Kiem closed the session by presenting plans to study the HIV reservoir after autologous and allogeneic transplantation and to maximise the engineering and engraftment of gene-modified HSPCs. Dr Kiem established a SHIV rhesus macaque latency model that allows the study of virus sanctuary sites and showed data on transplantation of HSPCs that express the C46 fusion inhibitor as one tactic to protect against viral infection. New methods using HSPC expansion drugs are being investigated to optimise genome editing conditions in primitive CD34+ HSPCs while retaining long-term progenitor potential and engraftment. In collaboration with industry partner Sangamo Biosciences, ZFN technology is also being applied to edit host restriction factors and/or mediate site-specific insertion of entry inhibitors, outcomes of these gene editing and HSPC transplantation approaches will be analysed using systems biology approaches.

Conclusion

As HIV cure research continues to become more mainstream at various conferences on HIV/AIDS, the Strategies for an HIV Cure Meeting remains the largest individual scientific conference focused exclusively on HIV cure research, with over 600 attendees on-site and over 300 additional viewers of the live-stream videocast. Since the inaugural meeting in 2012, much progress has been made towards our understanding of the persistent HIV reservoir and the development of curative strategies for testing in animal models and in pilot clinical trials. Although the ultimate goal of complete eradication of virus from the body still seems far away, recent examples of long-term remission in NHP models as a result of various immunological interventions have re-energised the field.

A key theme to emerge from the data presented at this year's conference was that long-term sustained remission of HIV infection may require a transient period of viral rebound of significant magnitude and duration prior to the establishment of immune control of viraemia to levels below detection with standard plasma viral load assays. The safety and ethics of TI where viral rebounds may reach levels of hundreds of thousands of copies of HIV RNA per mL for several days prior to suppression and control of viraemia by the immune system are important issues that will require continued discussion within the field among scientists, clinicians and community stakeholders.

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Conflict of interests

All authors declare no conflict of interest.

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