Progress Towards an HIV Cure: Update from the 2014 International AIDS Society Symposium

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Abstract

Biomedical research has led to profound advances in the treatment of HIV infection. Combination antiretroviral therapy (ART) now provides the means to readily control viral infection, and people living with HIV who receive timely and effective ART can expect to benefit from a life expectancy comparable to uninfected individuals. Nevertheless, despite effective treatment, ART does not fully restore the immune system and importantly HIV persists indefinitely in latent reservoirs, resulting in the need for life-long treatment. The challenges and limits of life-long treatment have spurred significant scientific interest and global investment into research towards an HIV cure. The International AIDS Society (IAS) 2014 Towards an HIV cure symposium brought together researchers and community to discuss the most recent advances in our understanding of latency and HIV reservoirs, and the clinical approaches towards an HIV cure under current investigation. This report summarizes and reviews some of the major findings discussed during the symposium.

Introduction

THE INTERNATIONAL AIDS SOCIETY (IAS) Towards an HIV Cure Initiative convened the 2014 Towards an HIV Cure Symposium on July 19–20, 2014 in Melbourne, Australia, immediately preceding the 20th International AIDS conference.

The symposium objectives were to (1) gather researchers and stakeholders to present, review, and discuss the latest research towards an HIV cure, (2) promote cross-disciplinary global interactions between basic, clinical, and social scientists, and (3) provide a platform for sharing information among scientists, clinicians, funders, media, and civil society.

The symposium, chaired by Françoise Barré-Sinoussi, Steven Deeks, and Sharon Lewin, brought together over 300 registrants, including virologists, molecular biologists, immunologists, clinicians, members of organizations of people living with HIV, and funders. The scientific gathering included both invited speakers (see Table 1) and a selection of oral and poster abstracts presenting the most recent advances in basic and translational science and clinical research.

In this symposium report, we summarize and review some of the key findings presented in Melbourne. Herein, a broad overview of the current research results that improve our understanding of HIV reservoirs and mechanisms responsible for HIV latency is provided, as well as the most recent clinical results and promising approaches towards an HIV cure.

Where Does HIV Persist on Antiretroviral Therapy?

The recent reports of viral rebound after prolonged remission for the “Boston patients”^{1,2} and the Mississippi child (referenced herein) raise the question about the localization of the source of rebound viremia during analytical treatment interruption (ATI; Box 1). More broadly, the characterization of the location of the HIV reservoir pool during antiretroviral therapy (ART) remains of primary importance. Brandon Keele and colleagues (NCI, United States) attempt to address this question specifically with a nonhuman primate model based on the development of a genetically engineered simian immunodeficiency virus (SIV) barcoded with 10 nucleotides (potentially >1,000,000 unique combinations).³ A high dose intravenous challenge with this virus is

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TABLE 1. INVITED SPEAKERS

<i>Speakers</i>	<i>Affiliations</i>
Jeffrey Lifson	Frederick National Laboratory for Cancer Research, United States
Geoff Hill	Queensland Institute of Medical Research, Australia
Melanie Ott	Gladstone Institutes and University of California, San Francisco, United States
Giulio Maria Corbelli	European AIDS Treatment Group, Italy
Daniel Kuritzkes	Brigham and Women's Hospital, Harvard Medical School, United States
Lars Østergaard	Aarhus University Hospital, Denmark
Jeremy Sugarman	Johns Hopkins Berman Institute of Bioethics, United States
Dan Barouch	Beth Israel Deaconess Medical Center and Ragon Institute of MGH, MIT and Harvard, United States
Jintanat Ananworanich	U.S. Military HIV Research Program, United States
Robert Murphy	Northwestern University, United States
Joseph Tucker	University of North Carolina, Project-China, China
Christine Rouzioux	Université Paris Descartes, France
David Kaslow	PATH, United States
Mike McCune	University of California, San Francisco, United States
Miles Prince	Peter MacCallum Cancer Centre, Australia

expected to lead to a systemic infection of rhesus macaques and the seeding of anatomic sites with genetically distinct viruses before viral suppression with ART. After 9 days of infection, up to 1,000 actively replicating variants were identified and high genetic diversity was maintained for 90 days. ART was further shown to be effective in decreasing viral load in this model. This unique ultradeep viral barcode is a promising tracking device for the viral source of recrudescence viremia upon ATI.

Sarah Palmer (University of Sydney, Australia), reported results from studies on memory CD4⁺ T cells isolated from different tissues from long-term virally suppressed subjects: peripheral blood, GALT, and lymph node.⁴ Cell-associated HIV DNA was detected mainly in memory CD4⁺ T cell subsets from all tissues analyzed. Longitudinal phylogenetic analysis by single genome amplification (SGA) showed expansions of identical HIV-1 sequences in all subjects analyzed at two time points. The majority of the identical sequences were enriched in the effector memory T cells (T_{EM}), the more differentiated subset of memory cells endowed with the highest proliferative capacity. Interestingly, one of the expanded sequences found in T_{EM} from one subject was replication incompetent indicating that cellular proliferation of this latently infected cell subset was responsible for the sequence expansion. All these results

BOX 1. THE ROLE OF ANALYTICAL TREATMENT INTERRUPTION (ATI) IN HIV CURE CLINICAL STUDIES

Once a research strategy has proven safe and encouraging, allowing participants to interrupt their antiretroviral treatment is crucial to assess the efficacy of that strategy in controlling HIV replication, as discussed during the roundtable on “Analytical Treatment Interruption in HIV Cure Research.”* There are now two different strategies that can be used: a fixed period—usually 16 weeks—of treatment interruption (traditionally identified as Analytical Treatment Interruption or ATI) or a Monitored Antiretroviral Pause (MAP) restarting ART at the moment of viral rebound. The absence of systematic ART reintroduction at viral rebound during the first strategy (ATI) allows a viral set point to be achieved. This strategy could be conceivable to assess whether the virological control of the immune system has improved or not by the given intervention tested. The second strategy (MAP) is considered safer for study participants and allows the assessment of the delay to rebound in the context of interventions aimed at reducing the HIV reservoir.

The type of treatment interruption should be specifically tailored depending on both the scientific question being addressed and on patient characteristics. ATI will be important to assess immunological interventions aiming to improve viral control, whereas MAP will be more appropriate to assess strategies aiming to reduce the reservoir size. The research trial design should also be adapted according to patient characteristics, as stressed by Giulio Maria Corbelli of the European AIDS Treatment Group. That is, some people are more willing to participate in these trials and can perform better, whereas more vulnerable people such as those with poor social or educational backgrounds present difficult challenges for the informed consent process. The education of participants is key in this early phase of research with real risks and low benefits for participants. Both types of intervention present some degree of risk that makes them extremely delicate from an ethical standpoint and it will be important to carefully manage risks and frustrations in case of lack of success.

Despite the ethical challenges, ATI is a crucial step in studies whose ultimate goal is to achieve viral control in the absence of ART. As stated by Lars Østergaard from Aarhus University Hospital, at the moment no reliable method has been validated to assess the predictive value of any *in vitro* test or parameter, one that could be benchmarked against a clinically relevant outcome. To minimize risks, research should aim for small, controlled interventional trials including as few patients as possible, using established standardized methods and very close and long-term monitoring.

*The panelists of the roundtable discussion on “The Role of ATI in HIV Cure Clinical Studies” were Edwina Wright (The Alfred Hospital, Monash University and Burnet Institute) (Chair), Giulio Maria Corbelli (European AIDS Treatment Group), Cynthia Grossman (NIH National Institute of Mental Health), Daniel Kuritzkes (Brigham and Women's Hospital, Harvard Medical School), Lars Østergaard (Aarhus University Hospital), Deborah Persaud (Johns Hopkins Children's Center), and Jeremy Sugarman (Johns Hopkins Berman Institute of Bioethics).

suggest that a significant mechanism of HIV persistence during ART could be driven in large part by cell proliferation. Further investigations are needed to characterize the functionality of such expanded HIV DNA.

Another strategy to address the location of the persistent cellular HIV reservoir is the identification of markers for latently infected cells. Two complementary approaches identify immune checkpoints, negative regulators of T cell activation, as biomarkers of HIV-infected cells during ART. Rémi Fromentin (VGTIFL, United States) reported a study performed with CD4⁺ T cells isolated from 48 subjects virally suppressed for at least 3 years. Using cell sorting and polymerase chain reaction (PCR)-based assays, the authors showed that PD-1 and LAG-3 identified central memory (T_{CM}) and transitional memory (T_{TM}) T cells enriched for integrated HIV DNA while TIGIT identified T_{EM} cells enriched for integrated HIV DNA.⁵ Importantly, combining the three cell surface markers further enriched reservoir cells. Longitudinal studies are needed to establish the selective advantage for a latently infected cell to harbor these markers and functional experiments will be performed to evaluate the therapeutic potential of blocking immune checkpoints.

The role of immune checkpoints in the establishment of a pool of latently infected cells was further demonstrated in work presented by Vanessa Evans (Monash University, Australia). Using their recently published *in vitro* model of latency,⁶ V. Evans *et al.* showed that PD-1 and Tim-3, another immune checkpoint, were preferentially expressed on resting CD4⁺ T cells that were latently infected following coculture with myeloid dendritic cells (mDC) *in vitro*.⁷ This suggests a likely role of immune checkpoints in not only favoring HIV latency as described by the study referenced above but also in promoting the establishment of latent infection in resting CD4⁺ T cells.

A key scientific priority for HIV research remains the development of assays to measure persistent HIV infection in order to evaluate the impact of curative interventions, identify the best candidates for ATI studies, and monitor patients in remission.⁸ Christine Rouzioux (Université Paris Descartes, France) and Nicolas Chomont (VGTIFL, United States) both presented overviews of the advancements and limitations of current culture-based and PCR-based assays. While the PCR-based assays are reproducible and relatively easy to perform, they may overestimate the size of the reservoir.⁹ Conversely, the quantitative viral outgrowth assay (Q-VOA) that measures replication-competent HIV may underestimate the size of the reservoir up to 60-fold.⁹

Nicolas Chomont presented a novel alternative assay, the Tat/Rev Induced Limiting Dilution Assay (TILDA), which measures the frequency of cells with multiply spliced HIV RNA (msRNA) upon maximal cellular activation with phorbol 12-myristate 13-acetate and ionomycin (PMA/iono).¹⁰ This assay may provide a more accurate representation of the frequency of reservoir cells previously estimated to be 60-fold higher than the Q-VOA,⁹ given that TILDA estimates the reservoir as 48 times higher than the Q-VOA and 6 to 27 lower than the PCR-based assays on the same sample set. Interestingly, TILDA allowed the identification of a latent reservoir in untreated HIV-infected subjects. The presence of latently infected cells in subjects naive of ART prompts the question of the potential benefit of initiating “shock and kill” strategies at the time of ART introduction.

Early ART to Limit the Size of the HIV Reservoir

The introduction of ART early during HIV infection is a current clinical strategy for limiting the size of the HIV reservoir. Deborah Persaud (John Hopkins Children’s Center, United States) reported an update on the Mississippi child who received treatment very early (at 30 h of age) for a period of 18 months. After a period of 27 months of remission in the absence of ART, the Mississippi child had a viral rebound preceded by a low increase in viral DNA in activated CD4⁺ T cells. The reintroduction of ART led to undetectable viremia and CD4 T cell recovery. This case emphasizes the early establishment of a latent, persistent HIV reservoir. The reservoir, as shown in this case, can persist for years in the absence of any HIV-specific immune response. Intensive investigations are currently underway to identify predictors of viral rebound in infants receiving very early therapy after perinatal infection. This case and that of the so-called “Boston patients” also emphasize that reducing the size of the HIV reservoir to an undetectable level alone is not sufficient to lead to a cure, and likely it will be necessary to also promote an effective immune response to tackle the virus that may rebound.

Rapid seeding of the viral reservoir was also reported by Dan Barouch (Ragon Institute, United States) in a nonhuman primate (NHP) study recently published.¹¹ After introducing ART at 3, 7, 10, and 14 days after intrarectal SIVmac251 infection of rhesus macaques, the authors found that the viral reservoir was rapidly seeded after infection and before evidence of systemic viremia.¹¹ Initiation of ART 3 days post-infection prevented the establishment of viral reservoir in peripheral blood but not in gut mucosa and proximal lymph nodes. In the absence of a virus-specific immune response, viral rebound during ATI was delayed by very early therapy but not eliminated.

Christine Rouzioux summarized several studies analyzing the impact of early ART.¹² The ANRS 147 Optiprim trial conducted in France shows that initiation of ART in the first 10 weeks of infection leads to a fast and continuous decay of the frequency of cells harboring HIV DNA in the peripheral blood. In addition, viral remission was achieved in two subjects who underwent ATI, indicating that early ART can induce posttreatment control. The posttreatment controllers, characterized in the ANRS Visconti cohort, present a reservoir mainly located in T_{TM} while the T_{CM} is the major contributor of the HIV reservoir for subjects treated during chronic infection. Early treatment appears to favor the establishment of a reservoir in cells with a shorter half-life and may accelerate reservoir decay. Finally, early ART initiation has previously been shown to reduce inflammation. Further investigations are needed to translate these findings to increasing the number of HIV remission cases. Additional strategies such as neutralizing antibodies and therapeutic vaccines need to be developed to achieve eradication in the context of limited HIV reservoirs induced by early ART.

Shock and Kill Latency Treatment

Among strategies to deplete the latent reservoir in patients on suppressive ART to achieve remission in the absence of therapy, new innovations for the “shock and kill” (also termed “kick and kill”) approach were presented at the symposium. The shock and kill strategy aims to treat patients

with compound(s) to shock or reactivate latent HIV proviruses to express viral proteins and virus particles.¹³ This may induce cell death either through virus-induced cytopathic effects and/or immune-mediated depletion via recognition of expressed viral proteins, leading to depletion of the latent reservoir in patients.

New approaches to shock latently infected cells

Multiple agents are reported to reactivate latent HIV in various *in vitro* latency models and *ex vivo* CD4⁺ T cells from patients on suppressive ART, with some promising candidates advancing to clinical trials.^{14,15} These agents include histone deacetylase inhibitors (HDACi) that remodel chromatin structure and make DNA accessible for the transcription of genes, including HIV proviral genes.¹⁴ Recent single or multiple dose clinical trials with the HDACi agents vorinostat or panobinostat both increased intracellular HIV RNA expression in patient CD4⁺ T cells. Moreover, panobinostat induced plasma virion release in 14 out of 15 patients and induced a reduction in proviral DNA in a subset of 15 patients,^{16,17} thus demonstrating for the first time that an HDACi shock strategy can lead to declines in the HIV DNA reservoir in some patients. Moreover, a clear association was observed between the panobinostat-induced reduction in proviral DNA and the time until viral rebound after a closely monitored ATI.¹⁶ Associations were also observed in patients between the reduction in reservoir size and immunology characteristics¹⁶ and understanding that these patient changes may be instrumental in finding optimal “kill” strategies following effective shock strategies.

Following the panobinostat trial, Ole Søgaaard and colleagues (Aarhus University Hospital, Denmark) presented new research from a recent phase I/II clinical trial of six ART-suppressed patients treated with multiple doses of a different HDACi, romidepsin.¹⁸ Romidepsin is currently approved for treatment of T cell lymphomas and more potently reactivates latent HIV *in vitro* than vorinostat or panobinostat.¹⁹ Treating patients with 5 mg/m² IV romidepsin 1 day per week for 3 weeks was essentially safe, with 34 mild grade one and 2 grade two (fatigue, fever) adverse events recorded. Cell-associated unspliced HIV RNA was induced in all patients and HIV plasma RNA (virions) increased from undetectable at baseline to readily quantifiable levels at multiple postinfusion time points in five of six patients. However, like other HDACi clinical trials, romidepsin did not diminish the HIV DNA reservoir in most patients except for one patient who also had the strongest induction of intracellular unspliced HIV RNA. Thus, while romidepsin can potentially shock latently infected cells into producing viral RNA, proteins, and virus particles, the failure to decrease the HIV DNA reservoir in most patients indicates that treatment with a single reactivation agent is unlikely to purge the latent HIV reservoir. Instead, combination therapies with agents that potentially reactivate HIV plus kill the reactivated cells will most likely be required to purge the latent reservoir in patients. Consequently, this research team is now testing romidepsin in a clinical trial with a therapeutic vaccine to prime the immune system to kill the infected cells reactivated by romidepsin and deplete the HIV reservoir.

In parallel to these encouraging results of the molecule romidepsin, it is important to bear in mind that HDACi may

also have negative effects on the “kill” step by affecting immune cells. Indeed F. Wightman *et al.* (Monash University, Australia) reported significant potentially adverse immunological changes including an increase in regulatory T cells without any significant change in HIV-specific T cells after cyclic dosing of the HDACi vorinostat.²⁰ These data emphasize that HDACi may have immunological properties that potentially hamper the possibility of subsequently killing the cells harboring reactivated virus.

In addition to romidepsin, new progress using alternative agents to more effectively shock latently infected patient cells was presented at the symposium.

Combining P-TEFb and NF- κ B activators. Gilles Darcis *et al.* (Institut de Biologie et de Médecine Moléculaires, Belgium) reported that combining two classes of compounds that activate two cellular proteins critical for efficient HIV transcription, NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) and P-TEFb (positive transcription elongation factor b), led to potent, synergistic reactivation of latent HIV.²¹ The first class of compounds, including prostratin, bryostatin, and ingenol, induces cellular protein kinase C (PKC) signaling leading to migration of active NF- κ B into the cell nucleus, which in turn promotes initiation of HIV mRNA transcription.^{15,22,23} The second class of compounds, including HMBA plus the JQ1, I-BET, and I-BET151 bromodomain and extra-terminal (BET) inhibitors, all release active P-TEFb in the cell, which in turn binds the HIV Tat transcriptional activator protein to drive elongation of HIV mRNA transcripts.^{24,25} Thus, both types of compounds drive different stages of HIV transcription to enhance HIV mRNA expression.

When a compound from either class was combined together, Darcis *et al.* found these combinations synergistically enhanced HIV gene expression and virion production. This synergy was observed in latently infected T cell and promonocytic cell lines, microglia, and, importantly, CD8⁺-depleted peripheral blood mononuclear cells (PBMCs) and resting CD4⁺ T cells from ART-suppressed patients. The combination of JQ-1 P-TEFb inducer and either prostratin, bryostatin, or ingenol NF- κ B activator had the best synergism in *ex vivo* patient cells.

Not all patient cells responded to these compounds, possibly due to the low frequency of latently infected cells in some patient samples precluding their response to these compounds from being measured in the study. However, when patient cells did reactivate, the combination of compounds led to synergistic reactivation in > 70% of responding patient cells. While prostratin, bryostatin, and ingenol had the undesirable effect of increasing activation markers on *ex vivo* patient cells that may lead to adverse toxic effects in patients, these compounds had low cytotoxicity in combination with JQ1 on cultured patient cells. Finally, flow cytometry of latently infected microglia or a T cell line demonstrated that the compound combinations reactivated a greater proportion of cells than the compounds in isolation. This implies that these combinations of agents would reactivate a larger proportion of latently infected cells in patients rather than just a few cells to a potent degree, and thus would be more beneficial to purge the latent reservoir in patients. Therefore, the JQ-1 P-TEFb inducer combined with either the prostratin, bryostatin, or ingenol NF- κ B activator appears promising to synergistically reactivate HIV expression in a greater proportion of latently

infected cells in ART-suppressed patients as part of a shock and kill therapy.

Benzotriazole derivatives. Vicente Planelles (University of Utah, United States) introduced an intriguing new class of benzotriazole derivatives that reactivate latent HIV in T_{CM} CD4⁺ cells, which are a long-lived reservoir harboring latent HIV in patients on suppressive ART.²⁶ Planelles *et al.* identified hydroxybenzotriazole (HBOt) as reactivating latent HIV using a drug screen in a primary T_{CM} cell model of HIV latency.²⁷ Expanded testing of other benzotriazole derivatives further identified 1-hydroxy-7-azabenzotriazole (HOAt) as reactivating latent HIV more potently than HBOt. HOAt combined with interleukin-2 (IL-2) reactivated latent HIV more potently than HOAt in isolation. Importantly, in two ART-suppressed patients tested thus far, HOAt reactivated virus particle production from their resting CD4⁺ T cells *ex vivo* using the quantitative viral outgrowth assay. HOAt was shown to reactivate latent HIV through sustained phosphorylation of the cellular transcription factor, STAT5 (signal transducer and activator of transcription 5), which binds the HIV-1 LTR promoter and activates HIV transcription in primary human CD4⁺ T cells.²⁸

Inhibitors of JAK and STAT5 impaired HOAt reactivation of HIV-1 latency. Thus, targeting factors controlling the JAK/STAT cell signaling pathway might be useful to reactivate latent proviruses in primary T cells *in vitro* in addition to the HOAt compound.

Importantly, HOAt has a second desirable attribute apart from reactivating latent HIV. Consistent with reports that benzotriazole derivatives have antiproliferative properties,²⁹ HOAt did not induce T cell proliferation, activation (measured by CD25 and CD69), or cytokine production. Thus, this new class of compounds with the highly advantageous attributes of reactivating latent HIV in patient resting CD4⁺ T cells without inducing cell activation, proliferation, or cytokines is an enticing new option for development in future shock and kill strategies.

New approaches to kill and clear reactivated latently infected cells

The results of recent clinical trials indicate that reactivating HIV-1 expression in latently infected cells with a single agent is not likely to be sufficient to eliminate these cells in most patients. In most ART-suppressed patients, administration of the single reactivation drugs vorinostat, romidepsin, or the antialcoholic disulfiram did not decrease the HIV reservoir, although panobinostat decreased the viral reservoir in a subset of patients.^{30–34} As such, an additional kill strategy will likely be required to eradicate reactivated cells in these patients. Therefore, new strategies to kill reactivated cells are coming under the spotlight and, encouragingly, the development of various kill strategies was discussed at the symposium.

Broadly neutralizing antibodies. Broadly reacting neutralizing antibodies (bNAbs) are generated in a minority of HIV patients but have the advantage of being more potent and of neutralizing a broader range of HIV subtypes than conventional Abs elicited by the majority of patients.³⁵ Thus, there is revived interest in trying to elicit bNAbs by vaccine

immunogens and/or infusing these bNAbs to either prevent or eradicate HIV infection in patients.

Dan Barouch (Ragon Institute, United States) reported that treating rhesus macaques with ART plus the PGT121 bNAb (which binds HIV envelope protein and blocks HIV interaction with cell receptors³⁶) decreased proviral DNA to undetectable levels and enabled virological remission after ART treatment interruption in three out of five rhesus macaques.³⁷ Thus, PGT121 depletes simian/HIV (SHIV) infection and achieves virological remission in a subset of animals, potentially via the broadly neutralizing and/or antibody-dependent cell cytotoxicity properties of this Ab. Therefore, administration of PGT121 has encouraging potential to diminish the HIV reservoir in ART-treated patients and may be useful in shock and kill eradication strategies to deplete reactivated latently infected cells.

Separately, the VRC01 bNAb potentially inhibits >90% of HIV strains through engaging the CD4 binding site on the HIV envelope (Env) protein, which blocks the binding of virus particles to cellular CD4 protein inhibiting cell entry.^{38,39} Infusion of this bNAb into macaques prevents infection by SHIV,⁴⁰ suggesting it may also prevent HIV infection in humans. Bin Su *et al.* (Inserm, France) reported that VRC01 bNAb prevented HIV-1 transmission from plasmacytoid dendritic cells (pDCs) to autologous CD4⁺ T cells in culture as well as cell-free viral transmission to CD4⁺ T cells.⁴¹ As pDCs link innate and adaptive immunity and transmit HIV to CD4⁺ T cells, VRC01 bNAb could be used to prevent viral transmission in patients. Additionally, this bNAb might also be useful as part of shock and kill eradication strategies to recognize HIV Env expression on reactivated infected cells to promote their clearance by antibody-dependent cellular cytotoxicity or other mechanisms⁴² analogous to PGT121 above.

Bispecific antibodies. Richard Koup *et al.* (NIAID Vaccine Research Center, United States) presented a novel approach to purge the latent HIV reservoir using a bispecific antibody to reactivate HIV from CD4⁺ T cells and then induce killing of these cells by recruiting CD8⁺ cytotoxic T cells.⁴³ Bispecific antibodies are artificial proteins composed of two separate antigen-binding fragments joined together at their non-antigen-binding ends. Here, Koup *et al.* linked an Fab, antigen-binding fragment that targets the HIV Env protein to a single chain variable fragment (scFv) that targets cellular CD3 (T cell receptor). The aim is for the Fab portion of the bispecific antibody to bind HIV Env expressed on target cells (predominantly CD4⁺ T cells) and the scFv portion at the other end of the artificial antibody to bind CD3 on all T cells, including CD8⁺ cytotoxic T cells, which can then kill the attached HIV Env-expressing CD4⁺ T cell. Thus these bispecific antibodies aim to enhance killing of HIV Env-expressing CD4⁺ T cells by recruiting any CD8⁺ cytotoxic T cell (irrespective of their CD8⁺ T cell epitope) to kill the attached virally infected CD4⁺ T cell, rather than relying on HIV-specific CD8⁺ cytotoxic T cells that are less frequent in patients.

T follicular helper CD4⁺ T cells (T_{FH}) in the germinal centers of lymph nodes (LN) are a source of residual virus replication during ART (see below) and contribute to the HIV reservoir. This is despite an expansion of follicular CD8⁺ T cells with cytotoxic potential in LN germinal centers in HIV⁺ individuals. However, adding HIV Env-CD3 bispecific

antibodies to sorted CD8⁺ T cells from LN germinal centers enabled specific killing of HIV-Env expressing target cells in culture. These results support the development of bispecific antibody therapy to kill HIV-infected CD4⁺ T cells in the LN of patients to purge the viral LN reservoir. As latently infected cells in ART-suppressed patients do not typically express HIV Env protein, these bispecific antibodies might best be administered with potent reactivation agents to induce HIV Env expression from latently infected cells for subsequent bispecific antibody recognition and killing by CD8⁺ T cells to purge these latently infected cells.

Increasing functional virus-specific CD8⁺ T cells for viral control. Rama Amara's presentation (Emory University, United States) further highlighted the importance of developing interventions to deplete infected T_{FH} cells in LN and mucosal germinal follicles by virus-specific CD8⁺ T cells to purge these reservoirs and control viral infection. Using a chronic SIV macaque infection model, animals with uncontrolled SIV infection were compared to animals vaccinated with an SIV DNA/MVA regimen to control infection. Uncontrolled, chronic SIV infection led to an enrichment of infected T_{FH} cells in LN and rectal sites that did not colocalize with CD8⁺ T cells.⁴⁴ Conversely, vaccinated macaques with reduced SIV loads had fewer infected T_{FH} cells in LN and rectal follicles, plus increased functional SIV-specific CD8⁺ T cells that colocalized with T_{FH} cells in tissue follicles.⁴⁴ This indicates that infiltration of virus-specific CD8⁺ T cells to LN and gut follicles limits virus-infected T_{FH} cells and enables viral control. Thus, inducing virus-specific CD8⁺ T cells via interventions such as bispecific antibodies or vaccination to clear virus-expressing T_{FH} and other CD4⁺ T cells reactivated from latency by shock compounds may enable the viral reservoir to be purged.

Autologous immune effector cells. David Margolis *et al.* (University of North Carolina, United States) proposed using patient immune effector cells to bolster the exhausted immune response in chronically infected patients to clear reactivated, latently infected cells.⁴⁵ Two options were presented: *ex vivo* expanded HIV-specific cytotoxic T cells (HXTCs) and cytokine-stimulated natural killer (NK) cells.

HXTCs were generated from patient T cells by sequential coculture with autologous dendritic cells, irradiated PHA-treated lymphoblasts, and finally irradiated K562 lymphoblasts all loaded with consensus, overlapping peptides for HIV Capsid (Gag), Pol, and Nef to expand HIV-specific cytotoxic T cells. This led to a 37- to 300-fold expansion of cytotoxic T lymphocytes in HXTCs, which contained >80% CD8⁺ T cells and 80% effector memory T cells. HXTCs inhibited autologous patient reservoir virus. Moreover, in a novel latency clearance assay modified from the quantitative viral outgrowth assay, HXTCs decreased virus recovered from vorinostat-reactivated resting CD4⁺ T cells from ART-suppressed patients, consistent with the HXTCs killing the vorinostat-reactivated cells. Thus, HXTCs have the potential to clear reactivated latently infected T cells in *ex vivo* patient cells and thus patients.

Ex vivo expanded cytotoxic T cells are safe and have been used to treat viral infections in oncology patients.^{46,47} They also allow the quantity and timing of HXTC administration to be accurately controlled in patients, a clear benefit if com-

BOX 2. ENGAGING RESOURCE-LIMITED COUNTRIES IN HIV CURE RESEARCH

Although the majority of people living with HIV reside in resource-limited settings (RLS), very few trials on long-term HIV remission are currently ongoing or planned in these countries. Indeed, among the trial categories of latency-reversing agents, therapeutic vaccines, gene therapy, and broadly neutralizing antibodies for HIV cure, only one is currently ongoing in RLS.⁵³ The roundtable discussion on "Preparedness for HIV Cure in Resource-Limited Settings"* addressed some of the specific issues related to RLS that must be taken into consideration. The panelists discussed how the responses and complication to interventions in RLS may be different from those in high-income settings and may be affected by specific host and environment factors, different HIV clades, and comorbidities. The scientific objective of limiting the seeding of the HIV reservoir may be addressed by early diagnosis, early treatment, and ensuring that patients remain virally suppressed. Although this approach may be technically possible, each of the above steps also represents a challenge in RLS. Nevertheless, although some of the HIV cure clinical approaches (e.g., gene therapy, stem cell transplantation) are difficult to transpose to RLS at present, other strategies such as treatment in early and acute HIV infection and pediatric studies remain feasible. Finally, the panelists also stressed the need for more social research and cost-effectiveness analysis in these settings.

*The panelists of the "Preparedness for HIV Cure in Resource-Limited Settings" were Paula Munderi (MRC/UVRI Uganda Research Unit on AIDS, Uganda) (Chair), Jintanat Ananworanich (U.S. Military HIV Research Program, United States), Robert Murphy (Northwestern University, United States), and Joseph Tucker (University of North Carolina Project-China, China).

binning with latency reactivation treatments. Although the affordability and scalability of this approach to treat large populations of patients seem complex, this proof of concept study is encouraging in indicating that this strategy could be effective in patients.

NK cells are immune effector cells in the innate immune system with a critical role responding to virally infected cells.⁴⁸ Isolation of autologous NK cells, prestimulation with IL-2 or IL-15 cytokines, and then coculture with vorinostat-reactivated resting CD4⁺ T cells from ART-suppressed patients decreased virus recovered from these cultures in the *ex vivo* latency clearance assay. This is consistent with NK-mediated killing of the vorinostat-reactivated cells. Thus, in addition to HXTCs, augmenting NK cell function using cytokines may also be a useful approach to help clear reactivated latently infected T cells in patients.

Therapeutic vaccination. Therapeutic vaccination is another strategy that could be used to improve the function of the immune system in ART-suppressed patients to deplete the latent HIV reservoir. Therapeutic vaccines could be used to stimulate HIV-specific cytotoxic T cell immune responses to deplete reactivated latently infected cells, decrease the latent

HIV reservoir during ART, and/or control viral replication following ART treatment interruption for sustained virologic remission.⁴²

Jeff Lifson *et al.* (NCI, United States) described the use of a cytomegalovirus (CMV) vector-based SIV vaccine in the SIV-rhesus macaque model to stimulate virus-specific immune responses. This CMV/SIV vaccine induced extremely broad CD4⁺ and CD8⁺ effector memory-biased T cell responses targeting novel SIV epitopes that were maintained indefinitely at widely distributed sites including mucosal effector sites. Moreover, this vaccine enabled $\approx 50\%$ of vaccinated monkeys to control SIV infection following intrarectal or intravaginal SIV mucosal challenge. This led to viral clearance over time in various tissue sites and failure to adoptively transfer infection to new animals, indicating clearance of the reservoir.^{49,50} Consequently, this CMV/SIV vector is now being tested for use as a therapeutic vaccine to boost viral-specific immune responses and to try to clear infection in macaques already infected with SIV and on ART (to mimic ART-suppressed HIV patients). Indeed, in macaques started on ART during early chronic SIV infection, subsequent administration of this therapeutic vaccine boosted CD4⁺ and CD8⁺ T cell responses specific to the SIV vaccine antigen. The impact on clearing the viral reservoir in these infected animals is currently under examination.

The ideal shock and kill purging treatment. Importantly, Anthony Fauci (NIAID, NIH, United States) in the subsequent 20th International AIDS Conference outlined what the ideal shock and kill purging treatment should entail to achieve sustained virologic remission off ART and justify changing patients from a one-pill-a-day ART regimen that currently controlled their infection. The ideal treatment should fulfill three requirements: be at least as safe as current ART, be simple and not require tertiary care, and be scalable to treat the millions of patients in need.⁵¹ While an ambitious goal in this relatively early phase of therapy development, these are important ideals for which the HIV cure field should aspire so that any effective treatment could be readily provided to the millions of patients in need of sustained remission off ART.

Latent HIV Suppression to Permanently Silence the Patient Reservoir

Rather than reactivating and killing latently infected cells to deplete the viral reservoir and achieve remission in patients, an alternative approach to achieving virological remission may be to permanently silence the latent reservoir, preventing viral rebound off ART. To permanently silence the latent reservoir, Melanie Ott *et al.* used a short hairpin RNA (shRNA) screen in the latently infected JLat cell line and identified three cellular proteins that repressed latent HIV transcription in the presence of strong reactivation stimuli: SMYD2, SUV39H1, and SETDB1.⁵² Follow-up studies revealed that SMYD2 acts on Tat methylation to regulate Tat activity and could be manipulated to keep HIV in a latent state despite cell activation to permanently silence the reservoir. Thus, permanent suppression of HIV latency is an alternative strategy that might also lead to remission off ART rather than the opposite approach of reactivating and clearing the latent reservoir via a shock and kill style strategy.

Conclusions

The 2014 IAS Towards an HIV cure symposium reflected the increasing scientific attention and funding in this field of research.⁵³

One of the overarching discussions focused on the terminology and definition of HIV “cure.” Recent results of viral rebound in the so-called Mississippi child⁵⁴ are a harsh reminder of the arduous task of complete eradication of HIV. As a consequence a “cure” for HIV infection, at least considered as a sterilizing cure with complete and definitive eradication of the virus, remains extremely challenging. Nevertheless, optimism stays high for the prospects of long-term viral suppression in the absence of ART, exemplified by the individuals of the ANRS Visconti cohort.⁵⁵ However, in light of the extreme complexity of achieving a full “sterilizing” cure, many symposium participants discussed the most appropriate terminology for the goal of long-term undetectable viremia in the absence of ART. Numerous proposals have been suggested, including “functional cure” and “sustained virological response” (echoing hepatitis C infection terminology). We propose that the terminology of sustained or long-term “remission” remains the most appropriate definition, in accordance with the terminology suggested by the NIH,⁵⁶ ANRS,⁵⁵ and others,⁴² all while retaining the aspirational goal of working towards an HIV cure.

During two intensive days of the symposium, scientists, clinicians, members of organizations of people living with HIV, and funders shared findings, thoughts, strategies, but also concerns. This meeting revealed the extent to which the scientific community has progressed in the development of tools to measure the HIV reservoir size and location, in the ability to assess mechanisms of HIV persistence during ART in human and NHP or murine models, in the development of promising novel curative agents for shock and kill strategies, and in the current testing of shock curative strategies in clinical trials. In this respect, the clinical outcome of future clinical shock and kill trials may be to show prolonged remission, which will require closely monitored ATIs. All the actors in this movement towards an HIV cure are eager and ready to work together to better integrate and involve resource-limited settings (Box 2), and to better communicate, inform, and educate people living with HIV to assist in moving towards a cure for HIV.

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