The latest science from the IAS Towards an HIV Cure Symposium 16–17 July 2016, Durban, South Africa

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Introduction

The 2016 edition of the International AIDS Society (IAS) Towards an HIV cure symposium was held in Africa for the first time, bringing together over 300 participants in Durban on 16–17 July 2016 to present and discuss the latest science in HIV cure and remission research. The symposium, chaired by Françoise Barré-Sinoussi, Steven Deeks and Sharon Lewin, included presentations by invited speakers (Table 1), roundtable discussion and oral and poster abstracts.

As the field of HIV cure and remission research grows and expands, the symposium covered virology, immunology, clinical studies and social sciences research, retaining its characteristic cross-disciplinary dialogue. As befits the location, an increasingly important proportion of participants, both researchers and community, were from sub-Saharan Africa. Out of the 65 invited speakers and panellists, 47% were women, echoing the positive trend of female researchers in HIV science. Over one and half days, the symposium was the place of discussion of the mechanisms of HIV persistence, and ways to reverse it, and the immunological responses to control and/or eliminate residual disease. Presentations included the latest scientific evidence on the clinical approaches towards cure and remission, as well as discussion on the perceptions and expectations of people living with HIV.

The symposium coincided with the launch of the International AIDS Society 2016 global scientific strategy: towards an HIV cure 2016 [1], a blueprint of the current and future research directions to be addressed to accelerate a cure and/or remission for HIV.

The keynote address was provided by Anthony Fauci who presented the challenges and opportunities in addressing HIV persistence [2]. Dr Fauci discussed how HIV persistence could potentially be addressed by two approaches: eradication of the reservoir (the classic 'cure') or the control of viral rebound by sustained virological remission. Among the strategies to address HIV persistence, Dr Fauci concentrated on discussing the control of viral rebound, defining it as 'sustained virological remission', indicating that the optimal starting point to achieve this status is a small reservoir and a competent immune system, which can be achieved by early initiation of combination antiretroviral therapy (cART). From that perspective, three possible strategies can be explored with the hope of achieving a state of sustained virological remission: natural immunity, therapeutic vaccines and passive transfer of HIV antibodies.

In the context of passive transfer of HIV antibodies, Dr Fauci presented the data from infusions of 40 mg/kg of VRC01 (a first-generation neutralising monoclonal antibody) 3 days prior to ART interruption and 14 and 28 days following. All of the 10 patients rebounded following ART discontinuation; nevertheless some variability from the moment of treatment interruption to time to rebound was observed. The median rebound time was 39 days, compared to a median 11–28 days in the absence of VRC01 treatment as per the literature, suggesting a modest influence, albeit transient. Analysis showed that rebound did not occur because the level of VRC01 fell below what is known to be the protective level. An analysis of the virus pre-infusion, however, showed that some of the individuals already displayed pre-existing resistance to VRC01 explaining the lack of extended time to rebound.

Overall these results show that multiple infusions of broadly neutralising HIV antibodies can be safe and well tolerated. To improve the length of sustained remission, further studies should explore antibodies with improved potency and breadth, antibodies with extended half-lives (which can be achieved relatively easily with minor modifications of the Fc region) and vector-based antibody production.

Paediatric HIV cure research

The 2016 Towards HIV cure symposium included a special session organised jointly with the 8th International Workshop on Pediatric HIV. The session was led off by Deena Gibbons and Nigel Klein who gave an overview of neonatal and infant immunology [3]. Dr Gibbons described her work on neonatal and infant CD4+ and CD8+ T cell responses that has led to the understanding that neonatal CD4+ T cells are not inert, just different, with production of IL-8 (CXCL8) rather than other cytokines, such as IFNγ, in response to stimulation. This may influence the earliest responses to HIV in exposed and infected infants. Dr Klein introduced the importance of the thymus and described differences in the impact of ART in paediatric *vs* adult HIV infection. In children, increased thymic output leads to a pattern of immune reconstitution fuelled by naïve T cells after starting therapy and it was noted that in the ARROW trial [4], giving ART before 2 years of age was associated with good CD4 cell reconstitution whereas in adults the pre-ART CD4 cell count generally determines the outcome on ART. In terms of virus persistence in children on ART, Dr Klein remarked that multiple factors, including the timing of ART initiation and varying exposure to immune activating stimuli during gestation or as neonates, may influence the size and composition of HIV reservoirs. Since there have been different outcomes of very early treatment in infants reported, with the most exciting being that of the Mississippi child [5], but others without the same sustained remission [6,7], Dr Klein and others have established the EPIICAL (Early-treated Perinatally HIV-infected Individuals: Improving Children's Actual Life with Novel Immunotherapeutic Strategies) project [8]. This consortium aims to identify immunological and virological predictors of the response to early ART in international cohorts as well as optimise methodologies to characterise correlates of viral control and test novel immunotherapeutic strategies. Hopefully, we will have exciting results from this collaboration in the coming years.

A roundtable discussion followed with several themes emerging [9]. These included the importance of studying cure-directed

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Table 1. List of names and affiliations of invited speakers and panelists

Invited speakers and panellists	Affiliation
Anthony Fauci	National Institute of Allergy and Infectious Diseases, NIH, USA
Gethwana Mahlase	ZimnadiZonke, South Africa
Andrew Phillips	University College London, UK
Deena Gibbons	King's College London, UK
Nigel Klein	University College London, UK
Paolo Rossi	University of Rome, Italy
Thanyawee Puthanakit	Chulalongkorn University, Thailand
Barbara Kingsley	HIV Activist, South Africa
Diana Finzi	National Institute of Allergy and Infectious Diseases, NIH, USA
Caroline Tiemessen	National Institute for Communicable Diseases, South Africa
Thumbi Ndung'u	University of KwaZulu Natal, South Africa
Olivier Lambotte	APHP Hôpital Bicêtre, France
Moses Supercharger Nsubuga	Joint Clinical Research Centre, Uganda
Jerome Singh	Centre for the AIDS Programme of Research in South Africa (CAPRISA), South Africa, and University of Toronto, Canada
Peter Newman	University of Toronto, Canada
Monique Nijhuis	University Medical Centre Utrecht, Netherlands
Elizabeth Connick	University of Arizona, USA
John Wherry	University of Pennsylvania, USA

interventions in the most heavily affected individuals and the importance of early treatment to limit reservoirs. Caroline Tiemessen commented that the impact of cure interventions may vary more widely in Africa with multiple different circulating virus strains and genotypes as well as environmental and social factors that may affect the response to treatment. A moving personal story from Barbara Kingsley, a South African HIV activist, also highlighted the importance of the goal of an HIV cure on an individual level and the strength of the community of persons living with HIV. In terms of early treatment, Thanyawee Puthankit described the shift in Thailand to now initiating ART for all infected infants by 3 months of age (with a median of 15 days from diagnosis) compared to in the past when ART was typically not initiated prior to 6 months of life. The positive impact of this earlier treatment was voiced by Paolo Rossi, not only in terms of potentially reducing the overall size of the HIV reservoir, but also resulting in a better immune response to standard childhood immunisations and preservation of memory T cell responses. Diana Finzi remarked that there is interest in funding strong proposals related to paediatric cure and that the recently funded Martin Delany Collaboratories include paediatric-specific components for the first time.

New insights into HIV persistence and rebound

Unravelling the mechanisms of HIV persistence is the key to curative strategies, and one of the proposed mechanisms is the constant replenishment of the viral reservoir by ongoing low-level residual replication that occurs despite ART. Giorgio Bozzi *et al.* [10] investigated whether HIV replication is ongoing in tissue compartments during cART by performing phylogenetic and compartmentalisation analyses of HIV populations in blood and anatomical compartments from three individuals who initiated ART shortly after HIV infection and maintained viral suppression for 8–16 years. No evidence of molecular evolution was detected in any tissue or in the peripheral blood, and clonal expansion of HIV-infected cells took place across multiple tissue compartments, the latter represented by identical hypermutated sequences. The authors concluded that no evidence of ongoing replication in tissues and peripheral blood could be observed.

For a better understanding of HIV-1 pathogenesis and persistence, and for the improved design of strategies towards the functional cure, it is important to identify biomarkers that could predict disease progression and the duration of post-treatment virological control. Alexander Pasternak *et al.* [11] quantified HIV-1 persistence markers in patients treated with temporary ART (24 or 60 weeks of treatment with a quadruple triple-class ART regimen) during primary infection. In the univariate analysis, both plasma viraemia and US (unspliced) RNA, but not MS (multiply spliced) RNA, were predictive for time to virological suppression (VS). In the multivariate Cox regression, US RNA at primary HIV infection was the only significant predictor of the time to VS. Upon ART interruption, all patients experienced virological rebound (VR) within 9 months. US RNA before ART interruption was identified as the only significant predictor of the time to VR. Further exploration of the potential of this biomarker as a predictor of post-treatment control in large-scale clinical trials aimed at HIV functional cure is warranted. Furthermore, the authors measured the same biomarkers at the virological setpoint (36 weeks after the therapy interruption) and assessed their predictive power for the disease progression (time to reach the CD4+ count of 350 cells/mm^3). Surprisingly, among the virological markers, MS RNA at virological setpoint after ART interruption was the strongest predictor of disease progression (much stronger than plasma viraemia), whereas US RNA was not predictive at all. Taken together, these data suggest that different mechanisms might drive HIV reactivation after therapy interruption and subsequent CD4+ T cell loss.

Immunology of HIV persistence

Elizabeth Connick presented an overview of her work on HIV persistence in lymph nodes and, more specifically, the B cell follicle [12]. She made several important points as previously published [13,14], including that CD4+ T cells within the B cell follicle have a 31-fold higher likelihood of carrying HIV RNA than those that are extrafollicular, that HIV replication appears greatest in the subset of CXCR5+PD-1low CD4+ T cells following *ex vivo* infection of tonsils possibly as a result of PD-1 downregulation after infection, and that, while not absolute, HIV/SIV-specific cytotoxic T lymphocytes (CTL) predominate in the extrafollicular rather than follicular zone. In new data from SIV-infected rhesus macaques, Dr Connick showed that monoclonal antibody-mediated CD8 depletion resulted in a large increase in extrafollicular SIV RNA with only minimal change in SIV RNA in the follicle. Only about 10% of tetramer-positive CD8+ T cells expressed the follicular homing phenotype characterised by expression of CXCR5 and lacking CCR7 and one potential strategy to promote entrance of CTL into the follicle could be to introduce CXCR5. As was pointed

out by Dr Connick, the data she described were all from untreated humans and macaques so delving deeper into this biology in ART-suppressed individuals is critical.

Ann Chahroudi *et al.* [15] presented findings using a novel model of SIV infection and ART in infant rhesus macaques. Infant rhesus macaques were orally infected with SIVmac251 and treated with the combination of tenofovir + emtricitabine + dolutegravir for 6–9 months with euthanasia scheduled while still on ART for an assessment of cellular and anatomic reservoirs. The data presented showed that ART was effective in suppressing viraemia to levels below the limit of detection in infant macaques and reduced cell-associated SIV DNA by 1–2 logs in peripheral blood. When subsets of CD4+ T cells were examined, naïve and central memory T cells contained the highest frequency of cells harbouring SIV DNA in blood and lymph nodes. Interestingly, when SIV RNA was measured by RNAscope in ART-suppressed compared to viraemic animals, lower levels were found in lymph nodes and spleen of ART-suppressed macaques, but levels of SIV RNA remained similar in the gastrointestinal (GI) tract and brain. Antiretroviral drugs/drug metabolites in tissues were measured by LC-MS/MS as well as IR-MALDESI (Infrared Matrix-assisted Laser Desorption Electrospray Ionization) revealing heterogeneous patterns in the lymph nodes and GI tract but very low or absent levels from the brain by these techniques. These findings have implications for future studies to assess virus persistence in infants as well as for the design of remission strategies in this population.

John Wherry gave a state-of-the-art closing lecture on the development and reversal of T cell exhaustion [16]. He reviewed the existing data on immune exhaustion in HIV infection and the impact of PD-1 blockade both in the setting of HIV and as cancer immunotherapy [17]. Understanding who will respond favourably to PD-1 blockade and who will not has been a focus in the oncology field and may be very applicable to HIV should these types of interventions move forward. Dr Wherry described that in patients with melanoma, anti-PD-1 reinvigorates exhausted T cells (TEX) and it is possible to predict who will respond to treatment by 3 weeks based on expression of specific markers on T cells. Delving deeper into the molecular mechanisms of T cell exhaustion and what happens when we try to reinvigorate these cells as well as the long-term effects of immune checkpoint blockade are critical factors likely to have an impact on favourable or unfavourable responses. As an example, chronic infection of mice with lymphocytic choriomeningitis virus clone 13 results in T cell exhaustion that can be reversed using PD-1 blockade, but this early response 'crashes' when mice are followed for longer periods of time with the transcriptional profile of T cells from PD-1 treated and controls becoming indistinguishable.

In some exciting and original work, Dr Wherry described epigenetic analysis of T cell subsets using ATAC-Seq, which utilises transposons to stick tags on regions of open chromatin. Using this technique, he described differences between TEX and naïve and memory T cells, with TEX being as different from memory T cells as Th1 are from Th2. He noted a unique enhancer element found only in TEX, but that reversal of exhaustion did not change much in terms of the enhancer landscape. However, there were about 650 unique changes in reinvigorated T cells with predicted transcription factor activity including NFkB, IRF1/2, and bZIP up with PD-1 blockade and NFAT, NR4A1, and EGR2 down with PD-1 blockade. The conclusion drawn from this work is that PD-1 blockade may allow TEX to re-access the epigenetic landscape of effector T cells, but that PD1-blockade alone does not completely reprogram TEX, such that a combination of checkpoint inhibitors may have a greater effect. These types of analyses are likely to have implications for remission strategies aimed at reinvigorating antiviral T cells in HIV-infected patients as part of the 'shock and kill' approach.

New approaches to kill reactivated latently infected cells

Studies to date show that the administration of single latency-reversing agents (LRAs) is generally not successful in reducing the viral reservoir [18,19]. While combinations of LRAs, including the LRA gnidimacrin in particular, may be more successful in this regard [20], additional strategies are required to kill reactivated latently infected cells. In addition to the immune-based therapies outlined below, new approaches to sensitise killing of reactivated cells were reported. Recent work by Cummins *et al.* [21] has shown that central memory CD4+ T cells, the primary HIV-1 reservoir, have an apoptosis-resistant phenotype favouring their survival, and possibly explaining their persistence despite reactivation of HIV-1 expression. Specifically, these cells express increased levels of the anti-apoptotic protein BCL-2. HIV-1 replication generates Casp8p41 that usually activates the pro-apoptotic protein BAK, however BCL-2 binds to Casp8p41 and prevents apoptosis. Venetoclax, a BCL-2 antagonist, was shown to reduce cell-associated HIV-1 DNA in *ex vivo* reactivated primary CD4+ T cells (including latently infected cells) from cART-suppressed HIV-1-infected individuals, indicating that venetoclax caused latently infected cells to die following reactivation. Cummins *et al.* [22] now report that venetoclax treatment of acutely infected CD4+ T cells *in vitro* reduced infected cell survival, cell-associated HIV-1 DNA and p24 production. Thus, BCL-2 antagonism may favour the death of acutely infected cells (reducing reservoir formation) and reactivated latently infected cells, while sparing uninfected cells. Pham *et al.* [23] report that enhancing HIV-1 virion tethering at the cell surface by BST2/tetherin to promote antibody-dependent cell-mediated cytotoxicity (ADCC) could be another means to eliminate reactivated latent reservoir. Broadly neutralising antibodies (bNAbs) have been proposed as a strategy to clear reactivated latently infected cells potentially via ADCC [24], however, HIV-1 limits exposure of ADCC-targeted epitopes through Nef- and Vpu-mediated CD4 downregulation and Vpu-mediated BST2 downregulation [25]. Pham *et al.* [23] showed that bNAbs efficiently induce ADCC, although with different potencies. They observed that, while CD4 downregulation limits ADCC mediated by CD4-induced specific antibodies (one class of bNAbs), BST2 depletion decreases envelope recognition and ADCC activity for most bNAbs. Correspondingly, upregulation of BST2 potentiates ADCC activity. Thus, strategies to restore virion tethering at the cell surface by BST2 will sensitise reactivated latently infected cells to ADCC by most bNAbs and represents a promising 'kill' strategy.

Immune-directed therapies: shock and kill

A number of immune-directed therapies are currently under study for their ability to reverse latency and/or enhance killing of reactivated latently infected cells, with some of these strategies having a double-edged effect. Pavlakis *et al.* [26] produced heterodimeric interleukin-15 (hetIL-15; the bioactive form of IL-15) and demonstrated that treatment with this cytokine in macaques significantly increased CD8+ T cells and natural killer cells with a cytotoxic commitment in tissues, including lymph nodes. Importantly, similar effects were observed for SIV-specific T cells. Furthermore, the presence of cytotoxic and actively proliferating effector CD8+ T cells in the germinal centres (an important HIV reservoir) of the lymph nodes was confirmed. Pavlakis *et al.* [26] suggest that hetIL-15 treatment may be used in combination with DNA vaccination (preferably a conserved elements vaccine [27])

to induce potent effector T cells that have access to virus sanctuary areas for virus eradication following ART interruption. Mylvaganam *et al.* [28] showed a dual effect of PD-1 blockade in destabilising the viral reservoir and enhancing viral control following ART interruption in macaques. PD-1+ CD4+ T cells form a significant proportion of the viral reservoir [29, 30], suggesting that PD-1 blockade may disrupt latently infected cells. Consistent with this, Mylvaganam *et al.* [28] observed transient and significant increases in viraemia in macaques on ART in response to PD-1 blockade. PD-1 blockade not only enhanced SIV-specific CD8+ T cell function prior to ART and accelerated ART-induced viral suppression, but it also significantly reduced viral load setpoint following ART interruption (up to 80-fold compared with pre-ART setpoint levels). Thus, PD-1 blockade in tandem with other therapeutic interventions and LRAs might enhance HIV eradication; although, John Wherry reported that reinvigoration of CD8+ T cell function (and the benefit thereof) by PD-1 blockade is temporary [16]. Other promising immune-based approaches with a double-edged effect on latency reversal and clearance of reactivated cells, include depletion of regulatory T cells (Tregs) [31] and administration of a dendritic cell-based HIV-1 vaccine [32]. Tregs impair CD8+ T cell function in chronic HIV-1 infection and may favour HIV-1 persistence [33]. He *et al.* [31] achieved significant Treg depletion in SIV-infected rhesus macaques who had supercontrol of SIV replication, resulting in both reactivation of SIV from the latent reservoir and boosting of SIV-specific CD8+ T cells that rapidly cleared reactivated virus. Dendritic cells also have the potential to reverse HIV latency [34]. Kristoff *et al.* [32] demonstrated both reversal of HIV-1 latency and promotion of HIV-specific CD8+ T cell responses by dendritic cells loaded with autologous HIV-1 antigen. Type 1-polarised dendritic cells are superior to type 2 in both the 'shock' and 'kill' of the latent reservoir and this highlights the potential of a personalised type 1 dendritic cell-based vaccine to target the reservoir.

The latest advances in novel cure strategies

Individuals who initiate ART during acute HIV infection (AHI) have a lower frequency of latently infected cells and could have a greater chance for viraemic control after treatment interruption (TI). Jintanat Ananworanich presented the results of a randomised study of vorinostat/hydroxychloroquine/maraviroc (VHM) plus ART *vs* ART alone given for 10 weeks, followed by TI at week 10 [35]. The study was conducted in adults treated in AHI (Fiebig III/IV) with high CD4+ counts and viral load (VL) suppressed to <50 copies/mL for >2 years. The VHM arm received three cycles of vorinostat 400 mg/day (14 days on/14 days off) plus hydroxychloroquine (400 mg/day) and maraviroc (1200 mg/day). VL was monitored weekly after TI. ART was resumed when confirmed VL>1000 copies/mL. Fourteen participants underwent TI (nine VHM plus ART, five ART) and all experienced VL rebound with no difference between arms (median: 3 weeks; range: 2–11 weeks). Time to VL rebound did not differ significantly to published chronic HIV cohorts, and ART duration, total HIV DNA in PBMCs, single copy VL, or CD4/CD8 ratio did not predict time to viral rebound. However, low-level plasma viraemia was increased in some VHM arm participants. Importantly, no acute retroviral syndrome occurred upon TI, no new resistance mutations could be registered by genotyping, and no virological failure occurred after ART resumption. Professor Ananworanich concluded that treatment in Fiebig III/IV with or without VHM did not result in delayed time to viral rebound and that alternative strategies to reduce or eliminate HIV reservoirs are needed.

Annemarie Wensing *et al.* [36] presented the EpiStem Consortium, aimed at guiding and investigating the potential for HIV cure in HIV-infected patients requiring allogeneic stem cell transplantation (ASCT) for haematological disorders, inspired by the case of Timothy Brown [37]. The project will systematically study samples from HIV-infected individual receiving ASCT aiming at quantification of the viral reservoirs, molecular and functional characterisation of infecting virus, and assessment of the dynamics of the innate and adaptive immune responses. So far, nearly 30,000 cord blood units in multiple European blood banks and more than 1,000,000 adult donors have been genotyped for CCR5 to generate a registry of CCR5Δ32 available donors. Twenty HIV-positive individuals with diverse haematological malignancies have been registered to the EpiStem cohort. Since 2012, 15 patients have been transplanted: five with a CCR5Δ32, one with a heterozygous, and nine with a CCR5 wild-type donor. So far, five patients have successfully passed the 12-month follow-up after transplantation, and eight patients have died after transplantation, despite achieving full donor chimerism in most cases. Preliminary analysis of virological and immunological data from blood and tissue samples shows a systematic reduction of HIV-1 reservoirs to very low levels, and in two cases with long-term follow up, the investigators were unable to detect infectious virus in blood independent of the CCR5 status of the donor. Based on the data, Dr Wensing hypothesised that the 'graft versus HIV-1 reservoir effect' contributes to the clearance of the viral reservoir. EpiStem is actively recruiting new cases and continues to systematically investigate HIV persistence over time to gain insight in potential HIV-1 eradication.

Gene editing of the CCR5 co-receptor locus in haematopoietic stem/progenitor cells (HSPCs) is a promising potential curative strategy for HIV. Chris Peterson *et al.* [38] presented the results of their study in the pigtail macaque (*Macaca nemestrina*) to interrogate the clonal persistence, trafficking, and antiviral efficacy of CCR5-edited cells. Recently, these authors demonstrated long-term engraftment of CCR5 gene-edited HSPCs [39]. The objectives of their current study were to understand how individual gene-edited HSPCs persist following autologous transplantation and virus infection, determine whether HSPC-derived, gene-edited progeny traffic to viral reservoir tissues, and develop strategies to increase the number of these cells *in vivo*. Zinc finger nucleases (ZFNs) were used to target the CCR5 locus in macaque HSPCs. Gene edited HSPCs were transplanted into animals either prior to infection with simian/human immunodeficiency virus (SHIV), or in SHIV-infected animals that are treated with a combination ART regimen designed to approximate a well-suppressed HIV-positive individual. Edited cells were measured in peripheral blood, bone marrow, GI tract, lymph nodes, and at necropsy in a panel of 25 tissues. The authors observed up to 14-fold enrichment of CCR5-gene-edited memory CD4+ T cells in SHIV-infected animals, consistent with virus-dependent selection against CCR5 wild-type memory CD4+ T cells. Gene-edited cells were found in a broad array of anatomical sites. These included tissues that were identified as viral reservoirs in their model, namely GI tract and lymph nodes. Spatial and temporal tracking of CCR5 mutations suggested that gene-edited cells persisted long term, and were polyclonal. This strategy resulted in stable engraftment of CCR5-mutated and SHIV-resistant HSPCs and their progeny in blood, and in tissues known to serve as viral reservoirs. Importantly, gene-edited CD4+ T cells underwent positive selection during active infection, further supporting the validity of this approach in the clinic. Transplantation did not increase the size of the latent SHIV reservoir. Their preliminary *ex vivo* homology-directed repair data suggested that these gene-edited cells could be engineered to undergo positive selection without the need for ongoing viral replication.

Monique Nijhuis *et al.* [40] presented an update of the use of CRISPR/Cas9 technology as a strategy for HIV cure. CRISPR/Cas9

is a complex of guide RNA (gRNA) and a Cas9 endonuclease that can cleave a target DNA sequence matching the gRNA. The resulting error-prone host repair introduces deletions or insertions (indels) that disrupt the function of the target DNA. CRISPR/Cas9 has been used to successfully deactivate HIV DNA in latently infected human cell lines [41], confer resistance of human cells to HIV replication *ex vivo* [42], and recently, in a proof-of-concept study using transgenic mice, CRISPR/Cas9 was demonstrated to have activity against integrated DNA in a range of cells and tissues *in vivo* [43]. Nijhuis *et al.* [40] investigated how HIV-1 might escape from CRISPR/Cas9. They designed gRNAs to target HIV-1 LTR, protease, reverse transcriptase, integrase and matrix regions. The CRISPR/Cas9 system was delivered to SupT1 cells using the lentivirus vector and the cells were subsequently infected with HIV-1 and monitored for viral replication. gRNAs differed in their potency to suppress HIV-1 replication (82–97%), however, rapid escape was observed with all gRNAs (even those targeting highly conserved regions), as also recently described by others [44, 45]. The host repair mechanism was found to facilitate the rapid escape, as some indels do not inactivate HIV yet allow escape from CRISPR/Cas9 recognition [44]. Importantly, Nijhuis *et al.* [40] demonstrated that a combination of potent gRNAs targeting different steps in the virus life cycle prevented viral escape – viral replication could not be rescued even after months of *in vitro* selection. A combination of gRNAs also improved the efficiency of preventing reactivation from latently infected cells transduced with CRISPR/Cas9 (>98% versus 40–95% for single gRNAs). Thus, a combination of CRISPR/Cas9 endonucleases, like combination ART, may be able to overcome the plasticity of HIV. Many challenges still remain with this technology, for example, the successful delivery to all latently infected cells *in vivo*.

Converging roads: HIV cure and cancer immunotherapy

Olivier Lambotte presented an overview on how oncology can help HIV cure strategies [46]. The precedent is very strong: the one and only patient in whom HIV seems to have been eradicated, received cancer treatment. However, this treatment is obviously too risky to be applied to every HIV-infected person, and other strategies have to be explored. Professor Lambotte emphasised that HIV cure and cancer cure strategies have the same goal: to kill or control rare events, and there are striking similarities between the biology of latently infected CD4+ T cells and quiescent poorly immunogenic cancer cells. Both latently infected CD4+ T cells and quiescent cancer cells are invisible for the immune system, and they both have intrinsic properties favouring persistence and survival, with defects of the immune system, and there is a deleterious role of the tissue microenvironment. Indeed, the cancer cells and their microenvironment are tightly dependent. In the first steps of the tumour evolution, the highly immunogenic cancer cells are killed by the immune system but progressively, because of intrinsic properties and because of various factors in their microenvironment, poorly immunogenic cancer cells will survive. During HIV infection a small number of latently infected cells will persist, despite ART, with many common mechanisms facilitating the persistence of the target cells. Most of the drugs used in the 'shock and kill' strategies come from oncology. In oncology, drugs used as LRAs are used in combination with other anticancer strategies and during a prolonged time. In HIV curative trials, if LRAs are used alone, the risk of failure is high because HIV-infected cells can survive, so there is a need to boost simultaneously the HIV-specific immune response by immunotherapies, including immune checkpoint blockers, therapeutic vaccine strategies, broadly neutralising antibodies, gene and cell therapies etc. Immune checkpoint blockers that control T cell activation have revolutionised cancer therapeutic strategies and are also promising in HIV curative trials, such as anti-PD-1 therapies. Professor Lambotte reiterated that it is the time to think about HIV cure as cancer cure: the common targets are identified and some weapons are already developed. However, there are some limitations that are specific to HIV, such as the patients' and physicians' acceptance of new treatments, and the feasibility of HIV remission strategies on a large scale.

Beyond biomedical research: ethics and social sciences in HIV cure research

Andrew Phillips highlighted the findings of a study he led on identifying key drivers of the impact of an HIV cure intervention in sub-Saharan Africa [47]. Using a model of HIV and ART to investigate the effect of introducing an ART-free viral suppression intervention in 2022 – using Zimbabwe as an example country – Phillips *et al.* found that interventions aimed at curing HIV have the potential to improve overall disease burden and to reduce costs. Phillips *et al.* argued that given the effectiveness and cost of ART, such interventions would have to be relatively inexpensive and highly effective.

Adam Gilbertson *et al.* [48] explored what, if any, social, psychological, and/or emotional benefits exist for HIV cure study participants, and how these factors may impact the responsible conduct of research. The study found substantial unanticipated social, psychological, and emotional benefits associated with HIV cure research, that are often the primary reasons participants continue to take part in HIV cure studies. Gilbertson *et al.* argued that such benefits should be considered when recruiting participants, and that they are important for informed consent processes.

Centred on African stakeholder perspectives on HIV cure research, Ciara Staunton *et al.* [49] reported that participants expressed some concern that the global North was driving the HIV cure agenda. Assessing and managing knowledge and expectations around HIV cure research emerged as a central theme in the findings. The study found that stakeholders felt that it was important to distinguish between biomedical and emotional cure, remission, and healing, and that avoiding curative misconception is critical. Treatment interruption was regarded as a major risk if 'cure' failed. Staunton *et al.* concluded that the synergistic effect of curative scientific research will be enhanced if the social and ethical dimensions of cure are taken into account.

Karine Dubé *et al.* [50] reported on the findings of their study on willingness to participate in HIV cure-related research among potential volunteers in the United States. Focusing on perceived personal and societal risks and benefits of participation in HIV cure research, the study found that increased cancer risk, developing resistance to ARVs, toxicities, and risks of stopping HIV medications, were the potential clinical risks most likely to discourage participation in HIV cure research among people living with HIV. The study found that the psychological benefit of feeling good about contributing to HIV cure research and gaining knowledge about one's health were the potential personal benefits most likely to motivate participation in HIV cure research. Dubé *et al.* concluded that understanding perceptions of risks and benefits is important to inform study design, informed consent, and recruitment and retention strategies for HIV cure-related research.

Conclusions

During the 2016 Towards an HIV cure symposium a diverse selection of the latest science around persistence and strategies

towards a cure or remission were presented. Among the new insights in this area of research, several themes emerged.

The terminology of cure remains one of much debate. As reported elsewhere, the participants of the symposium, discussed the use of sustained virological suppression, or virological remission as being more attainable goals in the mid-term [18]. However the concept of 'cure', intended as complete eradication of HIV, should continue to be explored and the terminology can be used as an aspirational goal. Significant growth in the number of groups active in behavioural and social sciences related to HIV cure is indicated by the significant increase in the number of presented abstracts (both oral and poster). The panel discussion around bridging social and biomedical sciences drew much attention and was the source of much debate and discussion with the audience, highlighting a desire and need to further explore this area. As suited to the location on the African continent, much discussion was held on the need to increasingly conduct HIV cure/remission research in resource-limited settings; members of both academia and community reminded the symposium participants that the facilities, infrastructure and patient population are present in Africa, and called for increased collaborations in the area. Finally, another emerging theme was that of the importance of cross-talk with other disciplines and other areas of biomedical research. In particular the 2016 symposium highlighted the notion of residual disease as being similar between HIV persistence and cancer, while indicating that many of the immune-based therapies currently being used for cancer are now being considered for use in HIV cure/remission studies. Collectively, the research presented at the symposium, together with the discussions, indicated that HIV cure research continues to grow and expand, gathering increasing attention from scientists working in related areas, and crucially, the global community, with a marked will to accelerate research towards a cure.

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