

Hepatitis B and HIV-1 2019 IAS cure forum: Lessons and benefits from interdisciplinary research

Item Type	Article
Authors	Paximadis, M;Patrigeon, S.P;Rajasuriar, R;Tatoud, R;Scully, E;Arbuthnot, P
Citation	Paximadis M, Perez Patrigeon S, Rajasuriar R, Tatoud R, Scully E, Arbuthnot P. Hepatitis B and HIV-1 2019 IAS Cure Forum: lessons and benefits from interdisciplinary research. J Virus Erad. 2019 Nov 4;5(4):234-244.
Publisher	Elsevier
Journal	Journal Virus Eradication
Rights	Attribution 3.0 United States
Download date	2024-10-03 14:59:50
Item License	http://creativecommons.org/licenses/by/3.0/us/
Link to Item	https://pubmed.ncbi.nlm.nih.gov/31754448/

Hepatitis B and HIV-1 2019 IAS Cure Forum: lessons and benefits from interdisciplinary research

M Paximadis^{1§}, S Perez Patriceon^{2§}, R Rajasuriar^{3§}, R Tatoud^{4*}, E Scully⁵ and P Arbutnot⁶

¹ Centre for HIV and STIs, Cell Biology, National Institute for Communicable Diseases and University of the Witwatersrand, Johannesburg, South Africa

² Division of Infectious Diseases, Queen’s University, Kingston, Ontario, Canada

³ Department of Medicine, Faculty of Medicine, University of Malaya and the Centre of Excellence for Research in AIDS (CERiA), University of Malaya, Malaysia

⁴ International AIDS Society, Geneva, Switzerland

⁵ Division of Infectious Diseases, Department of Internal Medicine, Johns Hopkins University, Baltimore, MD, USA

⁶ Wits/SAMRC Antiviral Gene Therapy Research Unit, School of Pathology, Faculty of Health Science, University of the Witwatersrand, Johannesburg, South Africa

[§]Authors contributed equally to the manuscript (in alphabetical order)

1 Introduction

The 2019 International AIDS Society (IAS) HIV and HBV Cure Forum took place on 20 and 21 July 2019 in Mexico City, Mexico, ahead of the IAS 2019 HIV Science Conference. Co-chaired by experts in both HIV and HBV fields, the Forum focused specifically on the intersection between the scientific efforts in hepatitis B virus (HBV) and HIV-1 cure research and sought to encourage interaction between these two disciplines to accelerate the pace of cure discovery in both fields.

Despite significant differences in HIV-1 and HBV clinical disease course, there are many similarities and shared challenges facing the development of a cure for each of the two infections (summarised in Box 1). The Forum aimed to bring together specialists from the two research fields to foster an exchange of ideas and highlight the benefits of interdisciplinary research. Research in both a HIV-1 and HBV cure share many similarities that range from basic science, pathogenesis and persistence to innovative therapeutic approaches. Identifying and quantifying viral reservoirs are both key aims for both infections, and, since both viruses have a reverse transcription step, there may be common therapeutic approaches either to eliminate the virus or to redirect immune responses.

The keynote address, Hepatitis B virus: an ancient curse and the modern blessing for its cure, delivered by Jake Liang (National Institutes of Health, Maryland, USA) reviewed most aspects of HBV molecular biology with particular emphasis on current and novel viral targets that may help advancing more effective therapies. The HBV treatment with licensed therapies currently includes six nucleoside/nucleotide analogues and two types of interferon whose limitations include:

- Rare loss of HBsAg-positive status in HBV carriers;
- Treatment achieves only partial reversal of hepatic fibrosis and inflammation;
- Decreased but not eliminated risk of hepatocellular carcinoma; and
- High cost and adverse effects of prolonged therapy.

Several new viral and host factors were described as potential targets for drug development with a key goal being the inactivation of the covalently closed circular DNA (cccDNA), the vitally important stable HBV transcription template. Advancing the understanding of the cccDNA formation and function is critical to providing the basis for effectively disabling this replication intermediate.

Box 1. Similarities and differences in HIV-1 and HBV infections and shared barriers to finding a cure

HIV-1	HBV
Differences	
Disease sustained by replication in T cells and macrophages	Disease sustained by replication in hepatocytes, i.e. hepatotropic
Primary infection rarely cleared	Acute infection often cleared in adults
Has a latent phase of infection	No latent phase of infection
No prophylactic vaccine available	Vaccine effectively protects against new infections
Unknown biomarker(s) for a functional cure	Biomarker(s) for a functional cure is known but limited
Similarities	
<ul style="list-style-type: none"> • Chronic infections are established • Virus replication cycle includes a reverse transcription step • Transmitted by unprotected sex, blood contact, and perinatal transmission • Current treatment controls viremia but does not lead to cure, which would require the eradication of HIV-1 provirus and HBV cccDNA • Sampling of peripheral blood may not represent all tissue reservoirs in HIV-1 infection and gives a limited view of the HBV reservoir in hepatocytes 	
Shared challenges to cure	
<ul style="list-style-type: none"> • Latency established in rare and/or long-lived cell populations • Risks of off-target effects of interventions aimed at eliminating integrated or episomal viral DNA • Viral latency established early in the course of HIV-1 disease and with unknown kinetics for HBV; early intervention is unlikely to eliminate the reservoir in either case • Difficulty in accessing tissue samples required for the study of persistent virus 	
HBV: hepatitis B virus; cccDNA: covalently closed circular DNA.	

Strategies to inactivate cccDNA include inhibiting host factors required for its formation such as TDP2 [1], disabling the HBx protein that affects the degradation of host restriction factors such as SMC5/6 that normally participate in cccDNA elimination [2] and the induction of APOBEC3A to degrade cccDNA [3]. Repurposing of existing pharmaceutical compounds as well as reverse and forward chemical genetics were proposed as methods to identify those that could lead to cccDNA inactivation. Such strategies would entail high throughput screening of small molecule libraries to recognise candidate drugs. Candidates with such capabilities are currently at an early stage of development. New advances are keenly awaited to understand their therapeutic potential.

The overview of the HBV replication cycle highlighted specific targets that may be considered for developing curative therapy. Efficient cccDNA disabling, which would eliminate the template

*Corresponding author: Roger Tatoud
Avenue de France 23, 1202 Geneva, Switzerland
Email: roger.tatoud@iasociety.org

for virus production is a focus of many HBV researchers, and, although achieving this crucial goal remains challenging, the field is poised to see significant progress in the near future.

Alternative targets for novel directly-acting anti-HBV agents include entry inhibitors such as Myrcludex B, capsid assembly disruptors (see summary of closing lecture) and HBV secretion inhibitors [4]. Gene silencing using synthetic siRNAs, gene and epigenetic modifiers with CRISPR/Cas and TALENs were also mentioned and will be discussed in more detail in later presentations.

2 Biomarker of cure – understanding persistence

A cure strategy will ultimately require HBV cccDNA eradication or permanent inactivation of integrated HIV-1 provirus. However, there remain gaps in our understanding of the drivers of cccDNA persistence in HBV infection and integrated provirus in HIV-1 and accurate measure and characterisation of cells harbouring them are presently significant barriers. In this Forum, progress in recognising and measuring these populations as well as the key remaining challenges were presented.

2.1 Measuring the HIV reservoir: insights from single cell analysis

Lilian Cohn (Chan Zuckerberg Biohub, San Francisco, USA) provided a comprehensive overview of recent advances in measuring HIV-1 latent reservoirs using single cell analysis. Strategies to characterise the viral reservoir and infected cells can be divided into two types: 1. nucleic acid-based assays which facilitate the analysis of viral/proviral sequences and HIV-1 integration sites, and 2. cell-based assays which characterise host transcriptome and protein expression of infected cells.

Studies identifying integration sites (ISs) have also been useful to analyse the clonal expansion of infected cells and the overall viral reservoir composition [5]. This data has also been key in understanding how specific ISs may modulate the HIV-1 proviral landscape and promote viral persistence. Several assays with various degrees of efficiency have also been developed to quantify intact integrated viruses (Box 2) [6–8].

However, the gold standard to quantifying replication-competent virus is still the *in vitro* quantitative viral outgrowth assay (qVOA) [9–12], which has been especially useful in our understanding of how clones of replication-competent viruses persist longitudinally in individuals on long-term ART [13].

Dr Cohn also presented an overview of cell-based assays, which help characterise infected primary cells: the HIV-1 Flow [14], HIV-1 SortSeq (Ho YC lab, unpublished) and the latency capture assays [15]. These use primary cells which are activated and then sorted for HIV-1 RNA (HIV-1 SortSeq) or cells expressing p24 (HIV-Flow) or HIV-1 *env* (latency cell capture, LURE) and characterised by flow cytometry. The advantage of these assays is their ability to simultaneously characterise other cellular and molecular features of primary infected cells. These include assay of surface receptor expression (HIV-Flow), gene expression (latency cell capture, HIV-1 SortSeq), integration site and HIV-1 splicing (HIV-1 SortSeq). However, these may have limitations related to the activation process, which may alter the cell phenotype.

Dr Cohn provided updates on technical advancements in single-cell RNA-sequencing (scRNA-Seq) techniques, which now enable high-throughput analyses and have the potential to significantly advance the field of HIV-1 cure research. The Seq-Well is a portable, low-input massively parallel scRNA-Seq platform, which allows robust single cell transcriptional analysis of peripheral blood

Box 2. Methodologies to measure the intact HIV-1 provirus

Method/approach	Readouts	Efficiency	Reference
Nested near-full length proviral DNA PCR amplification followed by viral genome sequencing	Frequency, sequence	Low throughput	[81]
Pair of qPCR primers quantify likely intact virus by digital droplet PCR (IPDA)	Frequency	High throughput	[6]
Near-full length PCR, digital PCR for four viral segments, nested PCR and sequencing	Frequency, sequence	Medium throughput	[8]

PCR: polymerase chain reaction; IPDA: intact proviral DNA assay.

mononuclear cells (PBMCs) from clinically acquired specimens in the order of 10,000 cells/sample [16]. This analytical approach was applied to samples from participants prior to and immediately after HIV-1 infection from the FRESH cohort and revealed important characteristics of the immune response dynamics in terms of cell types, status, interactions and associated drivers. Additionally, it has facilitated the critical understanding of cellular events associated with spontaneous control of infection in the absence of treatment. A potential challenge for using this approach is the sophisticated infrastructure and human capacity that are needed to deal with large datasets and the complexity of integrating sequencing data with available analytical techniques.

2.2 Seq-Well: portable, low-cost high-throughput RNA sequencing of single cells

Another challenge involves addressing the relevance of sequences of circulating virions found after rebound following treatment interruption to those found in infected cells. Prior work has suggested that intact proviruses identified from near full-length sequence analyses of circulating cells show approximately 40% overlap with viral sequences identified using viral outgrowth assays (VOAs), but no overlap with rebound viruses. The latter may, however, contribute to recombinant viruses found in blood [17].

Measurement of CD4 T cells in blood as a biomarker for cure is also complicated as those present in circulation make up only a minority of the total number of CD4 T cells in the body [18]. The limitations of monitoring CD4 T cells in blood must be weighed against the risks and limitations of more invasive sampling strategies.

The challenges in terms of biomarkers to monitor HIV-1 cure are comparable to those in HBV cure research where cccDNA is relatively inaccessible to sampling and the surface antigen (HBVsAg) expression does not confirm the presence of replication-competent virus. Researchers in both fields take into account that a reliable biomarker for cure will need to reflect true disease remission in tissue and not only in blood.

2.3 Clonal integration site expansion of infected cells is a main contributor of HIV-1 persistence in more differentiated T cell subsets during suppressive ART

Incomplete understanding of the drivers of viral persistence remains a major barrier in the field of HIV-1 cure. Numerous studies have reported that a large proportion of the viral reservoir is made of clonally expanded infected cells, which may arise from homeostatic proliferation, antigen-driven proliferation or survival advantage

conferred from proviral integration at specific sites in the host genome [7,11,19].

Jori Symons (Peter Doherty Institute, Melbourne University, Australia) explored the contribution of clonal expansion to HIV-1 persistence in different CD4 T cell subsets. In a cross-sectional cohort of 24 participants receiving suppressive ART (median 3.8 years), integration site analysis was performed in CD4 T cells sorted into naïve, stem cell memory, central memory, transitional memory, effector memory and terminally differentiated subsets. This analysis demonstrated that the contribution of clonal expansion to HIV-1 persistence increased with cell differentiation and that almost 60% of infected cells in the terminally differentiated subset were clonally expanded. Identical ISs were also found in multiple CD4 T cell subsets within a participant, implying that HIV-1 infected cells can differentiate while on antiretroviral therapy (ART). Additionally, larger clones were found more frequently in differentiated cells. Gene ontology and integrated pathway analysis showed evidence of a distinction between integrated genes and pathways in CD4 T cell subsets and single versus clonally expanded infected cells. In larger clones (>10%), HIV-1 ISs were found to be enriched in cancer genes, a result also consistent with the expansion resulting from homeostatic proliferation. This analysis also showed that HIV-1 integration in more differentiated cells frequently involved gene pathways associated with specific antigens, while single ISs more commonly involved chromatin structures that were inaccessible and in transcriptionally repressed genes. Collectively, this study suggests that mechanisms that maintain HIV-1 persistence in single and clonally expanded infected clones may differ.

2.4 Longitudinal proviral sequencing provides a window into selection pressures exerted on infected cells and provides an upper bound estimate of HIV-1 proviral half-lives which are influenced by expression and splicing potential

Work presented by Una O'Doherty (University of Pennsylvania, USA) examined selection pressures driving the HIV-1 provirus decay dynamics in participants on long-term suppressive ART. Current estimates do not distinguish the relative contribution of cell death and clonal expansion to those dynamics. To clarify this question the approach used involved counting clonal proviruses as a single provirus when first detected in longitudinal sampling.

The HIV-1 provirus sequences from four individuals receiving ART for over 10 years were analysed longitudinally. Consistent with previous studies, most were found to be defective with large deletions. Sequences were then divided into five categories based on the deletion or preservation of D1 and D4 donor splice sequence, which in a recent study were shown to play a critical role in positive and negative selection of intact and defective proviruses [20]. The D1⁺ splicing was reported to enhance HIV-1 protein expression while D4⁺ proviruses were associated with aberrant splicing. The abundance of each provirus category was then estimated over time and fitted into a first order decay model with either all clones counted or each clone counted only the first time it was detected. When all provirus clones were considered, sequences from the intact and nearly intact category declined faster compared to the 3' deleted (D1⁺D4⁻) one which decayed faster than the 5' deleted (D1⁻D4⁺) category and massively deleted (D1⁻D4⁻) proviruses. When clones were only counted once, 5' deleted (D1⁻D4⁺) proviruses contracted as fast as 3' deleted (D1⁺D4⁻) ones while massively deleted proviruses showed minimal turnover. This data suggested that 5' deleted (D1⁻D4⁺) proviruses associated with aberrant splicing may exhibit a greater propensity for clonal expansion and viral persistence compared to other categories of proviruses. Additionally, proviral

DNA decay appears to be most prominent in the initial 6 to 7 years after ART initiation before levels stabilise, reminiscent of the CD4 T cell reconstitution kinetics following ART-mediated viral suppression.

2.5 Immune activation markers associated with levels and diversity of intact HIV-1 proviruses during HIV/HBV co-infection

The final presentation of the session addressed how HBV may modulate characteristics of the HIV-1 reservoir in HIV/HBV co-infected individuals.

Xiao Qian Wang (Westmead Institute for Medical Research, Westmead, Australia) presented data on the clinical correlates of intact HIV-1 provirus isolated from circulating CD4 T cells of ART-naïve HIV/HBV co-infected ($n=19$) and HIV-1 mono-infected ($n=4$) individuals recruited in Thailand. A higher level of intact HIV-1 proviruses, analysed using the full-length individual proviral sequencing, FLIP assay, [21]) was found in both co-infected (7–80%) and mono-infected (23–59%) individuals compared to prior studies. However, both groups had proviruses with comparable genetic diversity.

Most participants had genetically unique HIV-1 proviruses and no associations were found between HBV markers (HBV DNA and HBsAg) and the frequency and diversity of intact proviruses in the co-infected individuals. However, higher levels of immune activation markers, specifically plasma CXCL10, sCD14 and lipopolysaccharides (LPS), were associated with increased frequency of intact proviruses while sCD14 and CXCL10 were significantly associated with enhanced genetic diversity of intact proviruses in the co-infected individuals. Similar associations were, however, not found in the mono-infected group. Studies are now ongoing to explore the impact of HBV on the HIV-1 reservoir 3 years post-ART initiation. Understanding how HBV interacts with the establishment and persistence of HIV-1 following ART is critical given their overlapping epidemics.

2.6 HBV cure therapies: how do we measure success?

John Tavis (Saint Louis University School of Medicine, USA) provided an overview of the issues of assessing the success of HBV cure therapies. A functional HBV cure is a stable state after therapy with sustained loss of HBsAg from blood, with or without seroconversion with the presence of HBs antibodies, and the likely persistence of intrahepatic cccDNA but not in blood. This is thought to indicate sufficient viral immune control to prevent disease progression in the liver, although the virus may re-emerge in the presence of deficient immune control [22]. A clinical definition of this state is, however, still lacking because of the lack of reliable HBV biomarkers.

The major hurdle facing HBV cure is the reactivation of replication as a result of persistent cccDNA at undetectable levels, this may occur after viremia clearance during acute infection. The persistence of cccDNA is demonstrable by the resurgence of disease in the presence of immune suppression.

Of note, HBV can also integrate into the host DNA and, although it cannot support viral replication, integrants may produce HBV proteins, including the surface antigen [23]. Current HBV biomarkers in terms of disease course (Box 3) include HBV proteins and antibodies to HBV proteins that provide evidence of HBV replication and immunity, each with their caveats. Crucially, there is presently no reliable method to detect cccDNA, the most important indicator of persistent HBV infection. There is, however, consensus that ideal biomarker characteristics for HBV cure should: 1. report on the presence of cccDNA; 2. be non-invasive, e.g.

Box 3. Current biomarkers to measure HBV disease course

Biomarker	What it is	What it means
HBsAg	Viral surface protein	HBV infection or transcriptionally active HBV integration
HBsAb or anti-HBs	Antibodies to HBsAg	Effective immune response following infection or HBV vaccination
HBcAg	Viral capsid (core) protein	HBV infection
HBcAb or anti-HBc	Antibodies to HBcAg	Past or present HBV infection
HBeAg	Secreted variant of HBcAg	High HBV replication
HBeAb or anti-HBe	Antibodies to HBeAg	Low HBV replication
HBV DNA	Viral genome in virus	Active viral replication

HBV: hepatitis B virus.

serum derived; 3. be amenable to point of care testing; and 4. be affordable [24].

The loss of HBsAg is currently the best indicator of HBV cure, although this marker may be unreliable for several reasons. First, a patient may eliminate cccDNA but produce HBsAg from integrated viral DNA. Second, HBsAg tests are not sensitive enough to detect trace levels of cccDNA. Finally, despite HBsAg loss, replication may be reactivated from residual cccDNA, if viral immunity is not established or weakened by immunosuppression.

Other viral proteins including HBeAg, HBV core antigen (HBcAg), polymerase and X proteins are also poor candidates as biomarkers as they are either present at too low concentrations or may be lost as a result of mechanisms other than cccDNA clearance [25].

Dr Tavis introduced potentially useful new HBV biomarkers during the Forum. The HBV core-related antigen (HBcrAg) includes HBcAg, HBeAg and p22cr, a processing variant of HBcAg and is more readily detectable in blood. Data suggest a correlation with cccDNA function, but its detection is still limited by sensitivity issues and validation studies are still ongoing.

Other potential markers include HBV RNAs which are secreted in virion particles. They are excellent biomarkers of functional cccDNA as they reflect production of essential HBV products such as pgRNA, HBcAg, polymerase and HBsAg. Early results from validation studies indicate a good correlation between HBV RNA and cccDNA.

Although promising, these new potential biomarkers may be unreliable on their own and will need to be combined with others to assess HBV cure. A significant challenge in the field is understanding which combinations are most informative. Studies addressing this question are often hampered by a relatively small number of patients who have achieved functional cure on current treatment regimens.

The immune system is also seen a critical component to achieving HBV cure. There are many lines of evidence that support this notion such as the protective effect conferred by anti-HBsAg, either through natural clearance or immunisation, and the potential to relaunch HBV infection from a single cccDNA copy when immunity wanes.

Therefore, achieving an HBV cure will depend on the ability to establish immunity against the virus. Whether this can be achieved

through enhancing innate or adaptive immune responses remains unknown and is discussed extensively in a later section (see section 5.1 Immunotherapy: vaccines and antibodies). It is not yet clear whether immune control, once established, can be a reliable biomarker of HBV cure.

3 Role of antivirals in HIV-1 and HBV cure strategies

Global HIV-1 eradication remains elusive with 1.8 million new infections in 2017, often associated with late diagnosis, gaps in linkage to care and incomplete viral suppression. The importance of a multipronged approach was emphasised with the need to improve ARV tolerability and convenience, thus maximising adherence as well as optimising pharmacokinetics and tissue distribution. These features are key to pre-exposure prophylaxis (PrEP) effectiveness, a strategy known to prevent HIV-1 transmission that impacts on both its incidence and prevalence [26].

Prolonged ARV half-life offers a way to improve adherence and is a focus in drug development (Annual Rev Med 2019). Data on MK-8591, a highly potent long-acting new ARV studied as a parenteral formulation in rats and non-human primates, were presented by Dr Hazuda (Merck and Inc., USA). Studies have shown that when delivered with an implant, it is concentrated for over 180 days in the tissues associated with viral transmission and replication, such as the gastrointestinal tract. Dr Hazuda discussed how p24 detection in tissue could be a much more sensitive marker than the p24 assay in blood and used for treatment monitoring. The drug could be available orally as a daily, weekly or monthly dose.

Massimo Levrero (University of Lyon, France) discussed the difficulty of defining HBV cure. He noted that there are many barriers to HBV cure when using currently available drugs in the context of cccDNA stability, integrated HBV DNA and a defective immune response to the virus in chronic carriers, none of which are directly addressed with current therapeutic strategies.

A functional HBV cure can be defined as an asymptomatic status and undetectable HBsAg and HBV DNA in blood but with the presence of cccDNA in liver. A complete cure would include unmeasurable cccDNA in the liver but with, potentially, the presence of integrated DNA. No evidence of HBV infection, including an absence of integrated viral DNA into hepatocyte genome, would be considered as a sterilising cure.

To achieve HBV cure, cccDNA stability and defective antiviral immune responses need to be considered. Evidence suggests that cccDNA half-life is about 4 months and whether the pool is influenced by non-cytolytic elimination or hepatocyte turnover remains unclear. However, and as described above, there is no reliable way to measure cccDNA without carrying out a liver biopsy.

New antiviral drugs are being developed to address these challenges and to eliminate cccDNA by preventing its synthesis or increasing its degradation. Many options were presented at the Forum, such as ccc_R08, a first-in-class orally available cccDNA destabiliser that has achieved sustained HBsAg and HBV DNA reduction in the HBV circle mouse model. Increasing hepatocyte turnover would also enhance cccDNA degradation but would be contraindicated in cirrhosis. Capsid inhibitors prevent virus assembly (see later) and could work as an intensification strategy, together with cccDNA destabilisers.

The other options presented were inhibitors of HBsAg release or HBV gene silencers aimed at suppressing HBsAg expression or cccDNA transcription. A 'shock and degrade' strategy for HBV

cure similar to the ‘shock and kill’ approach in HIV-1 cure strategies is also being explored, although it may be limited by the infection burden in hepatocytes. Agents would be initially used to reactivate the virus and followed by the administration of cytokines and other small molecules to induce degradation of cccDNA and other viral DNA. This has parallels to the approaches under investigation in HIV-1, but will require a thorough assessment as HBV infection in hepatocytes may be substantially higher than in HIV-1 latently infected T cells.

Overall, there is a strong interest in cure research based on use of antivirals for both HIV-1 and HBV diseases. For HIV-1 this could involve using very long acting ARVs to achieve a functional cure and for HBV, a complete cure may be achieved by successfully disabling cccDNA.

4 Advancing gene therapy to treat patients infected with HIV-1 and chronic carriers of HBV

Currently available technology, particularly in the field of gene editing, now potentially provides the means for an HIV-1 and HBV infection cure. Approaches include direct virus targeting as well as developing cellular resistance to infection.

4.1 Gene therapy for HIV-1

Pablo Tebas (University of Pennsylvania, USA) presented recent progress in advancing HIV-1 gene therapy. Gene engineering of autologous CD4 T cells or CD34⁺ hematopoietic stem cells to block HIV-1 infection has been a popular approach. The method uses strategies that involve harvesting cells from patients, manipulation and selection *ex vivo* followed by reinfusion [27]. Valuable information for developing this technology was derived from the understanding of processes underlying the cure of Timothy Brown, the ‘Berlin patient’.

Although it generated considerable enthusiasm, a caveat remains in as far as bone marrow transplants from patients with a *CCR5* delta32/delta32 mutation are not always successful [28]. Nevertheless, disabling *CCR5* through gene therapy has been commonly used in an attempt to prevent HIV-1 infection of CD4 T cells.

In addition to using gene editing methods (CRISPR/Cas, transcription activator-like effector nucleases [TALENs] or zinc finger nucleases [ZFNs]), expression of *CCR5*-silencing short hairpin RNAs (shRNAs), TAT activation response (TAR) decoys and inhibitors of viral entry have also been tested [29].

The first clinical trial using ZFNs to mutate *CCR5* was reported in 2014 [30]. Results showed that, after analytical treatment interruption (ATI), the viral rebound and set-point correlated with the frequency of *CCR5* mutation in CD4 T cells. Modest improvement of engraftment of modified cells could be achieved by including cyclophosphamide treatment. Despite low numbers of engineered cells in treated patients, improved HIV-1 specific immunity of *CCR5*-modified cells is likely to provide a survival advantage. However, an important limitation of this approach was that the treatment did not affect viral reservoirs.

Engineering cells to produce antiviral proteins (AVPs) is another strategy that looks promising [27]. The AVPs may be secreted and typically act by preventing HIV-1 cellular infection by HIV-1. Examples include antibody-encoding sequences, which importantly do not need to be expressed from non-hematopoietic cells to provide HIV-1 resistance.

Promising results from preclinical evaluation of using CRISPR/Cas to target HIV-1 proviral sequences directly, together with

ARVs [31], were also presented. The procedure entailed sequential administration of long-acting slow-effective release antiviral therapy (LASER ART) followed by delivery of *LTR*- and *gag*-targeting CRISPR/Cas using recombinant serotype 9 adeno-associated viral vectors (AAVs). Proviral sequences appeared to be completely removed from some of the HIV-1-infected humanised mice, as rigorous analysis failed to demonstrate viable HIV-1 in tissue from the animals showing a good response. This observation is important as it provides a proof of principle that gene editing may inactivate HIV-1 reservoirs and augurs well for gene editing as an approach to curing HIV-1 infection.

An indirect benefit to gene therapy has been derived from decades of intense research on the biology of HIV-1 replication. Resulting information provided the detailed understanding needed to develop the now widely used replication-defective and safe recombinant lentiviruses. These vectors retain the property of integrating proviral sequences into infected cells, which may be harnessed to achieve sustained expression of therapeutic transgenes. Lentiviral transduction has been particularly important for advancing CAR-T cell therapy, which has rapidly gained popularity in cancer [32] and antiviral therapy [33]. Indeed, lentivector-transduced T cells are currently being developed to counter HIV-1 itself [34].

4.2 Gene therapy for HBV

Man-Fung Yuen (University of Hong Kong and Arrowhead Pharmaceuticals, USA) presented a study involving clinical evaluation of gene silencing as a mode of HBV treatment. The candidate drug, ARC-520, comprises HBV-targeting short interfering RNAs (siRNAs) and a hepatotropic peptide that facilitates delivery and endosomal escape of the siRNA in liver cells [35]. Clinical trial data have shown a reduction of HBsAg in viral carriers, with a particularly impressive effect in HBeAg-positive patients. Multiple ARC-520 administrations produced several log reductions in many of the markers of HBV replication, especially HBV DNA. Flexibility of the approach was confirmed by design of alternative siRNAs that are effective when the target site is disrupted in integrated DNA. It remains to be established whether the siRNA-based approach will be curative and possibly synergistic with other treatment modalities.

Approaches using gene editing to cure HBV infection are not as advanced as for HIV-1 therapy. Most studies, summarised at the Forum by Patrick Arbuthnot (University of the Witwatersrand, South Africa), have been based on preclinical evaluations.

A major advantage of using gene editing to treat HBV infection is that the technology provides the means to mutate and permanently disable the problematic replication intermediates including cccDNA (reviewed in [36]).

Predictably, there has been a plethora of articles describing the use of CRISPR/Cas to mutate cccDNA (reviewed in [36]). Many of the studies have demonstrated inhibition of HBV replication in cultured cells, but compelling evidence for direct targeting of cccDNA has not always been present. Using CRISPR/Cas technology to inactivate cccDNA following systemic administration of vectors encoding antiviral gene editors may also be complicated by pre-existing immunity to the Cas9 proteins of commensal *Streptococcus pyogenes* and *Staphylococcus aureus* bacteria [37,38]. Because systemic *in vivo* administration of vectors encoding gene editors is required for HBV therapy, immunity to the Cas9 proteins may attenuate antiviral activity. This problem is not as significant for HIV-1 gene editing approaches that entail *ex vivo* manipulation.

In addition to CRISPR/Cas against HBV, TALENs have been shown to be effective in murine models [39,40].

As proteins derived from plant bacteria, TALENs obviate the problem of pre-existing immunity. However adaptive immunity may well compromise efficacy following repeated administration of these gene editors. To improve specificity of action, unpublished work was presented that showed good efficacy of TALENs that function as obligate heterodimers.

Another approach that shows promise involves TALEN-derived silencers to induce epigenetic changes of HBV sequences without causing cleavage of DNA. Despite evidence indicating that gene editing provides the means to disable cccDNA permanently, evaluation in preclinical models that closely simulate HBV replication will be an important precursor to clinical evaluation. Specificity of action, efficient hepatotropic delivery of the therapeutic nucleic acids and scalable drug production will be vital for the successful implementation of gene editing to treat HBV infection.

5 Immunotherapy

5.1 Immunotherapy: vaccines and antibodies

Immunotherapy is a therapeutic approach that targets or manipulates the immune system to harness the host's innate and adaptive immune responses to bring about the elimination of pathogens and(or) diseased cells [41].

Both HIV-1 and HBV infections are characterised by immune dysregulation and, because of their shared routes of transmission, approximately 10% of HIV-1 positive patients worldwide are thought to be co-infected with both viruses [42]. Immunotherapy involving vaccines as well as antibody-based approaches is an avenue being actively investigated in the field of HIV-1 and HBV cure research.

5.1.1 Broadly neutralising antibodies as components of an HIV-1 cure strategy

The discovery of the 'next-generation' anti-HIV-1 broadly neutralising antibodies (bNAbs), some of which have shown to have extraordinary potency and very wide breadth *in vitro*, has sparked renewed interest in their potential for HIV-1 prevention and treatment through passive immunisation.

Katherine Bar (University of Pennsylvania, USA) presented a convincing case for the use of bNAbs in HIV-1 cure based on pre-clinical and clinical data generated in the short period since the availability of the 'next-generation' bNAbs. Their ability to suppress viral replication by neutralisation of cell-free virions, potential for Fc-mediated clearance of virus-infected cells and eliciting of a vaccinal effect through immune complex formation, together with their long half-life and relative safety make them highly attractive compounds for advancing cure strategies [43].

Dr Bar reported on preclinical studies in chronically simian HIV (SHIV)-infected rhesus monkeys. Using bNAbs as mono or dual therapy, virus suppression, immune-modulatory effects as well as a reduction of proviral DNA in tissue reservoirs were demonstrated [44,45], thereby setting the stage for human clinical trials testing.

A bNAb that targets the viral CD4 binding site, VRC01, was tested in individuals that had initiated ART during chronic [46] or acute infection [47] in separate trials. Although both studies reported only a modest increase in time to rebound following ART interruption, the chronic ART-initiation trial resulted in a significant increase in VRC01-resistance, whereas no change in sensitivity was observed in the acute ART initiation one. No prior testing for virus sensitivity to VRC01 was undertaken in either of these trials.

Three infusions of two bNAbs (3BNC117 and 10-1074) and pre-screening for viral sensitivity [48] showed a more prolonged viral suppression (median of 21 weeks) after ART interruption in antibody-sensitive individuals. Antibody-resistant viruses did not emerge and two individuals showed viral suppression long after both bNAbs had been cleared (>30 months).

Another study with SHIV-infected rhesus macaques passively infused with the same two bNAbs during acute HIV-1 infection resulted in sustained suppression of viremia in a subset of animals. The effect could be attributed to potent CD8 T cell immunity [49] thereby reinforcing the idea that early treatment with bNAbs can enhance cellular and humoral immunity [50].

Finally, a passive infusion of a bNAb (PGT121) together with vesatolimod, a latency reversal agent, toll-like receptor 7 (TLR7) agonist were administered to SHIV-infected rhesus macaques that had received ARVs during acute infection. A subset of treated monkeys (5/11) [51] did not show viral rebound following ART cessation. These animals had undetectable viral DNA in PBMCs and lymph nodes and failed to transfer infection to naïve hosts following adoptive transfer of PBMCs and lymph node mononuclear cells. Furthermore, viral loads remained undetectable following CD8 T and NK cell depletion. Although this study makes a convincing argument for the efficacy of bNAb administration combined with innate immune stimulation, a caveat is that it represents an idealised system of hyper-acute ART in non-human primates.

'Next-generation' bNAbs, which have been engineered to improve their therapeutic properties, i.e. with enhanced breadth, effector function and extended half-life were also discussed. Several clinical trials involving bNAbs in various combination strategies are currently in progress and many more are planned that will provide valuable information for future cure strategies.

An example of such bNAbs (BiA-SG) was the subject of the talk presented by Dr Mengyue Niu (University of Hong Kong, HK). Previous work of this group has shown that as an engineered tandem bi-specific bNAb, it displayed substantially improved breadth and potency. When administered using an AAV vector, it showed complete viremic control and elimination of infected cells in a humanised murine model [52]. Dr Niu presented new unpublished data showing that BiA-SG fully protects Chinese rhesus macaques from a lethal dose of SHIV. A single, early therapeutic administration can prevent rapid disease progression and leads to sustained virological control in the presence of strong humoral and CD8 T cellular responses. Although further studies addressing the pharmacokinetics, tissue distribution and antibody development of this new class of molecules will need to be carried out [53], their performance in human clinical trial settings is awaited with great interest.

5.1.2 Multiscale imaging of therapeutic antibody distribution and localisation

Thomas J Hope (Northwestern University, Chicago, USA) delivered a captivating talk on multi-scale imaging of therapeutic antibody distribution and localisation. Fluorophores conjugated to antibodies allow the direct visualisation and quantification of intravenously administered antibodies in plasma, tissue and mucosal secretions [54].

Therapeutic antibodies currently represent the fastest growing biotech sector, however very little is known about their body distribution following an infusion [54]. Dr Hope focused specifically on how function-enhancing modifications can dramatically alter their body distribution *in vivo*. He described how the VRC01 LS mutation, a change in the Fc fragment made to prolong the antibody half-life, alters its pattern of early distribution in primates.

Wild type VRC01 accumulated in the liver and small intestine, while the LS variant was concentrated in highly vascularised tissues such as the heart, lungs, uterus and colon. However, by 1 week after administration both antibodies had similar distribution. This technology will provide much needed insight into the mechanisms mediating antibody distribution to specific anatomical sites, which will be immensely valuable in advancing the wide use of therapeutic antibodies.

5.1.3 Ad26 and MVA therapeutic vaccines in acutely treated HIV individuals

Therapeutic vaccination against HIV, HBV and other pathogens aims to reprogram the host's immune response to allow for better control of viral replication in the absence of therapy [55]. Dr Donn J Colby (Thai Red Cross AIDS Research Centre, Bangkok, Thailand) presented data from a trial in which the Ad26/MVA HIV-1 vaccine (recombinant adenovirus serotype 26 prime with a modified vaccinia Ankara boost – US Military HIV-1 Research Program) was tested as a therapeutic vaccine in 18 acutely ART-treated individuals with nine participants in the placebo arm.

Although the vaccine proved to be safe and immunogenic in terms of humoral, ADCC, CD4 and CD8 T cell responses, it failed to lead to ART-free viremic control. The median time to viral load rebounds (>20 HIV-1 copies/ml) was 20 days, with no occurrence of an acute retroviral syndrome or new resistance mutations. Data analysis to characterise biomarkers that could predict viremic rebound are ongoing. Dr Colby concluded by suggesting that a TLR7 agonist in conjunction with the vaccine may be more successful. In future trials it may be more informative to allow for longer ARV interruption to accurately assess the post peak viral load set-point.

5.1.4 Restoring or replacing adaptive immunity in hepatitis B

Turning the focus to HBV, Dr Mala Maini (University College London, London, UK) gave an up-to-date, detailed overview of why and how an immunotherapeutic approach towards a functional cure for chronic hepatitis B (CHB) should and could be tackled.

Dr Maini suggested that the rationale for an immunotherapeutic approach towards an HBV cure is based on the knowledge that most infected adults manage to resolve HBV infection and maintain residual virus under successful long-term immune control. Also, even when HBV is chronically established, it remains susceptible to immune control. Although the focus of Dr Maini's presentation was on the restoration of the anti-HBV adaptive immunity, she mentioned the importance of intrinsic/innate immunity as a key stage where therapeutic interventions may be applied. Such interventions include hepatocyte-targeted interferon- α modifications, LTR β , RIG-I, LXR and TLR-7/8/9 agonists as well as the use of immunomodulatory cytokines like IL-12 (a cytokine able to rescue exhausted CD8 T cell responses [56]).

The generation of a complex repertoire of virus-specific B and T cells with helper or cytotoxic effects during HBV infection is considered of paramount importance and ultimately determines the outcome of infection [57]. Therefore, CHB can be viewed as an immunological disorder [58], which leads to multiple humoral and cellular immunity defects. Dr Maini illustrated how studies have shown that in individuals with CHB a subset of regulatory B cells mediates anti-HBV CD8 T cell response suppression through IL-10 secretion [59]. Also, although HBsAg-specific B cells have been found to persist in blood and liver, they were found to have defective antibody production in patients with CHB [60].

With an emerging role for humoral immunity in chronic HBV infection control, Dr Maini suggested that it is imperative to

identify and target molecular constraints of B cell immunity as part of a functional cure. It is also well established that in CHB, virus-specific T cells are depleted and functionally defective and that this exhaustion state is a key determinant of viral persistence [61].

Dr Maini elaborated how multiple mechanisms are at play to constrain HBV-specific T cells. These include the high quantity of antigenic peptides and duration of exposure to antigen load, the up-regulation of multiple co-inhibitory receptors (including PD-1, LAG-3, Tim-3 and CTLA-4), transcriptional, metabolic and epigenetic defects, negative regulation by NK cells and suppressive cytokines.

Therefore, tailored therapeutic approaches are required to boost virus-specific T cell responses. These can include therapeutic vaccination, approaches to reduce HBV antigen production (e.g. by RNA interference), use of immuno-regulatory cytokines (e.g. IL-12, INF- α), checkpoint blockade alone or in combination with therapeutic vaccines, use of mitochondrial antioxidants to reverse T cell mitochondrial defects and careful timing and manipulation of liver NK cell immunity to block their pathogenic effects but enhance their protective functions [62].

Other immunotherapeutic approaches entail 'replacement of the endogenous adaptive immunity' by: 1. adoptive transfer of genetically engineered T cells that can either express a canonical HLA class I restricted T cell receptor (TCR); 2. administering chimeric antigen receptor (CAR-T cells) [61]; and 3. using therapeutic monoclonal or bi-specific antibodies.

Lastly, Dr Maini discussed how optimising the immunogenicity of therapeutic vaccines can be achieved by careful selection of the patients and strategic and rational vaccine design. Boosting HBV immunity will be a trade-off between immunity and immunopathology since the liver injury is immune-mediated. Because CD8 T cells mediate both protection and liver injury, hepatic flares are likely to be an inevitable result of immune boosting. A question that arises is whether the promotion of non-cytolytic responses together with controlled hepatocyte lysis to eliminate integrated DNA can be achieved. Dr Maini concluded by stating that efficacy of HBV-directed immunotherapy will require rational combinations of immunological and virological approaches for the various patient groups.

5.2 Immunomodifying agents

5.2.1 Engineered immune-mobilising monoclonal T cell receptors against viruses

The session focusing on therapies aimed at the direct modification of immune responses to mediate virus eradication was opened by Dr Lucy Dorrell (University of Oxford, Oxford, UK). She reviewed the challenges facing curative HIV-1 immunotherapy including minimal expression of viral antigens, immune evasion, inefficiency of methods to induce viral transcription, immune epitope escape, inaccessibility of sanctuary sites to cytolytic effectors, and cells that may be resistant to CD8 T cell-mediated killing. She then presented data on engineered immune-mobilising monoclonal T cell receptors (TCRs) against viruses (ImmTAVs). Researchers have sought to redirect immune responses with soluble mediators such as bi-specific antibodies and chimeric-antigen receptor T cells (CAR-T cells) [63–65]. The ImmTAV molecules are an alternative approach, engineered from a T cell receptor with specificity for an HIV-1 target antigen in soluble form linked by a CD3 activating antibody fragment (anti-CD3 scFv). When an antigen is processed and presented on the appropriate type of HLA molecule, the ImmTAV will bind the free anti-CD3 scFv and engage nearby cytolytic T cells, irrespective of specificity, to activate full effector

function. The TCRs are antigen selected, cloned, affinity matured to reach high levels of binding efficiency and stabilised with interchain disulphide bonds. The approach seeks to leverage the convenience of soluble molecule manufacturing with the full range of intracellular antigens and efficient killing offered by T cell recognition. The platform has been developed for cancer immunotherapy [66,67] and has multiple infectious disease applications in development.

Promising proof of concept from the first HIV-1 *gag*-specific ImmTAV was presented showing activity at low effector-to-target ratios when tested *ex vivo* with CD4 T cells from HIV-1 positive patients on ART [68,69]. Additional work will, however, be necessary to define the levels of viral antigen expression required in the reservoir for the approach to be effective, and evaluate whether the technology can indeed be translated across HLA types and assess potential toxicity.

5.2.2 Immunomodulators

Two brief presentations highlighted attempts to target HIV-1 reservoirs within lymph nodes (LN). Extending their recent work on FX1, an inhibitor of the transcription factor B cell lymphoma 6 (BCL6) [70], Dr Yanhui Cai (Wistar Institute, Philadelphia, USA) presented data on the effects of FX1 on germinal centre (GC) formation. In prior studies, they had demonstrated that inhibiting BCL6, a transcription factor critical for T follicular helper (Tfh) cells and function of other activated T cells, led to reduced HIV-1 infection *ex vivo* [70]. FX1 has been demonstrated to reduce GC formation in mice [71], thereby highlighting its potential use to limit privileged sanctuary sites harbouring HIV-1. In a rhesus macaque model, FX1 treatment reduced lymphoid hyperplasia, Tfh CD4 T cells and their precursors, markers of proliferation and BCL6 expression in GCs. The authors suggested that this type of approach may be useful for combination interventions that include recently described heterodimeric interleukin-15 (hetIL-15) agents. These compounds have been reported to enhance the activation and localisation of CD8 T cells in B cell follicles in LN [72], which addresses one of the challenges of directing potent effectors to sanctuary sites.

Maria Pino (Yerkes Primate Center, Emory University, Atlanta, USA) presented a study on the use of fingolimod at the time of ART initiation. This compound is a sphingosine-1-phosphate receptor modulator used in multiple sclerosis [73,74] that promotes the retention of several types of lymphocytes within lymphoid tissue. Authors used this agent to address whether retaining lymphocytes in LN would enhance contact between infected cells of the reservoirs and cytolytic effectors, and whether they could provide insights into the relative contribution of different sites (peripheral blood versus LN) to the reservoir. Twenty-two rhesus macaques were infected with SIV and started ART 6 weeks later to allow reservoir seeding. Fingolimod was then administered along with ART for more than 8 weeks. In eight of the animals, ART was continued for an additional 10 months when it was interrupted and animals followed to determine time to viral rebound. As expected, fingolimod induced peripheral lymphopenia. Interestingly, this did not influence plasma viremia decay kinetics, suggesting that it is not dependent on CD4 T cell recirculation. A SIV DNA and SIV RNA decline was noted using DNAscope and RNAscope in LN of both fingolimod-treated and ART alone groups. A subset of animals treated with Fingolimod had a slight delay in viral load rebound and lower viral set-point. These findings were associated with lower amounts of SIV DNA in follicular T helper cells. Work to define why there are differences in cell phenotypes is ongoing. These studies may help to identify which features associated with sanctuaries such as tissue localisation,

cell type preference and(or) cell subset programming are the most critical barriers to HIV-1 eradication.

Lucy Dorrell also presented data of the initial studies working towards the development of ImmTAV molecules with HBV antigen-specificity. These have identified a potent and specific molecule for an HBV-specific ImmTAV cell with *in vitro* results showing elimination of targets expressing HBV antigens.

The features of immune dysfunction in CHB provide distinct challenges. Dr Garg from Gilead Sciences offered a broad overview of challenges facing immunomodulatory therapy. Specifically, current treatments block viral replication in CHB but do not inhibit HBV surface antigen production. As discussed elsewhere in this article, numerous features of the immune response are dysfunctional in CHB with a low frequency of antigen specific T cells, high rate of exhaustion of HBV-specific T cell responses and dysfunctional B cell responses. Some of these features are likely to be directly linked to chronic exposure to high antigen levels and specific tolerogenic liver environment [75]. While ImmTAV offers one type of approach to addressing these issues, another possibility to consider is immunomodulation directed at boosting endogenous responses. In order to do so, Dr. Garg presented data on the use of an agonist of the innate immune sensor, TLR8. This immune sensor has a broad distribution of expression and is found in monocytes, macrophages, dendritic cells, neutrophils and regulatory T cells. Stimulation induces IL-12p70 but not IFN γ and *in vitro* studies shows induction of IFN γ , reduced PD-1 expression on CD8 T cells and lower levels of regulatory T cells. These observations suggest a profile of increased effector capacity in response to TLR8 activation. Small animal models are limited as TLR8 is nonfunctional in mice and rats, but testing in the woodchuck hepatitis model achieved lower levels of HBV surface antigen associated with IL-12 induction, without a type-1 IFN response. Direct TLR stimulation to enhance immune responses has also targeted TLR7 in the past. Data at the meeting supported this approach in HIV-1 cure efforts.

Sharon Riddler (University of Pittsburgh, Pittsburgh, USA) presented a Phase 1B randomised, blinded, placebo-controlled, dose-escalation study on the safety and biological activity of GS-9620 (vesatolimod), a TLR7 agonist. In the context of HIV-1, activation of innate immunity has been suggested as a pathway to stimulate viral expression and to enhance control of stimulated virus. The TLR7 agonists were shown to activate multiple immune cells and stimulate virus expression in SIV-infected rhesus macaques on ART [76]. In these studies, declines in measures of HIV-1 reservoir and sustained viral control after interruption of ART were observed in a subset of monkeys [76]. The TLR7 agonism in combination with therapeutic vaccination were associated with lower viral DNA levels, improved virological control and delays in rebound in SIV-infected rhesus macaques. In addition, combination with bNAb infusion in a SHIV-1 infection model led to delayed viral rebound and absence of detectable virus after treatment in a subset of monkeys [51,77].

Earlier studies on healthy volunteers had demonstrated the safety of the agent at doses ranging up to 12 mg with detectable induction of interferon-stimulated genes at doses above 2 mg [78]. The current study enrolled 48 HIV-1 positive individuals who were randomised to receive vesatolimod at escalating doses (peak of 10mg x 3 plus 12mg x 7). There were no grade 3 or above adverse events related to the study drug or drug discontinuations as a result of adverse events. Vesatolimod induced CD69 expression on NK cells along with increases in interferon-stimulated gene 15 (ISG15) and circulating cytokines (interferon gamma-induced protein 10 [IP-10], interferon-inducible T cell- α chemoattractant [ITAC], and interleukin 1 receptor antagonist

[IL-1RA]) in some participants at doses above 6 mg. There were no consistent increases in plasma viral load above 20 copies/mL or evidence of changes in HIV-1 RNA by single copy assay or in cell-associated HIV-1 DNA and HIV-1 RNA. Further studies will be necessary to define the reasons for the differences between these results and the NHP studies. The authors reported that ongoing trials are evaluating the efficacy of vesatolimod alone or in combination with other therapeutic agents.

In another intersection between efforts aimed at HIV-1 and HBV cure, Dr Garg presented data on the use of checkpoint inhibitor blockade to counter HBV infection. Given the high rates of PD-1 expression among HBV-specific T cells [79], checkpoint inhibitors offer means to achieve functional responses. Enthusiasm for this approach has been somewhat tempered by the potential risk of activating T cell responses against a highly expressed antigenic target, but results from a dose escalation cohort in individuals with advanced hepatocellular carcinoma offer some insight [80]. Overall, these results showed that anti-PD-1 therapy was generally safe and well tolerated with significant virological responses in a subset of participants, including one who showed HBsAg loss. These data provide some support for boosting endogenous immunity as a means of achieving an HBV cure.

For both HIV-1 and HBV infections, immunomodulatory therapies continue to be a balance between harnessing highly effective responses and limiting significant toxicities. Novel approaches to achieve these goals offer both the opportunity to move closer to a cure and the chance to define critical pathways that maintain chronic infection.

6 Achieving a cure: the science and beyond

The panel discussion was opened with an impassioned speech delivered by Moses ‘Supercharger’ Nsubuga (‘Stigmaless’, Uganda) who has been living with HIV-1 since 1994. Moses outlined 10 reasons why he believes people living with HIV-1 need a cure (Box 4)

As a community advisory board member, Mr Nsubuga stressed the need to simplify the language of science to inform communities affected by HIV of the efforts made to find a cure. He also emphasised that conferences give people hope and community advisory boards (CABs) should be empowered with ‘cure knowledge’ to inform people on ground level. There are many potentially dangerous misconceptions about an HIV-1 cure that need to be addressed (Box 5) through much needed educational activities and increased participation of community representatives at conferences and other training programmes.

Barriers to achieving a cure for both HIV-1 and HBV infections were discussed and included:

- lack of an appropriate animal models for these infections;
- absence of biomarkers in the cure field;
- host variability and various viral genotypes/sub-genotypes that require identification of the appropriate populations for evaluating therapeutic candidates and ethical considerations arising from testing cure therapies in humans;
- barriers between companies, academic institutions, consortia, funding agencies and regulators impede accelerating research towards a cure;
- communicating research in terms accessible to a range of stakeholders from those affected to medical practitioners, regulators and the public in general;
- the need to engage with pharmaceutical companies to invest in development of research tools (biomarker, models); and
- the absence of a roadmap to cure.

Box 4. Benefits of an HIV cure: a community perspective

Addressing treatment-related concerns

1. Lifetime adherence to medication is challenging, especially when there are many pills and competing life challenges
2. Side-effects of medications experienced by many
3. Intersection of HIV-1 with poverty and food insecurity means that taking ART with food as instructed is not always possible

Addressing access concerns

4. Sustainability of ART and care funding in sub-Saharan African countries
5. Frequent ART stock-outs in resource-limited settings
6. Inaccessibility of ART for many people living with HIV in resource-limited settings
7. Increased resistance to available drugs, and lack of treatment options

Addressing social and political perception associated with HIV-1 infection

8. Stigma associated with being HIV-1 positive and associated beliefs that one is going to die, infect others and unable to contribute to society
9. Limitations to employment opportunities as a result of HIV-1 positive status
10. Political instability in sub-Saharan African regions resulting in substantial movement of people between countries and lack of information about these individuals’ HIV-1 status

ART: antiretroviral therapy.

Box 5. Common community misconceptions about an HIV cure:

- Complete viral suppression is a cure and people may stop taking their ART once viral loads are undetectable
- The ‘West’ has a cure, for example Timothy Brown, which is being concealed from Africa
- Patients can stop taking ART since witchcraft, pastors and prophets can cure HIV
- Conspiracy theories, for example funders, advocates and pharmaceutical companies seem to be ‘quiet’ about cure
- A cure is coming by 2030 (UNAIDS 90-90-90 target)

ART: antiretroviral therapy.

As we consider the development of a cure, a thorough understanding of what ‘cure’ means is important, in particular to dispel some of the myths. There is a danger to assume that the research community understands what is required; there is a need for better communication within and across communities (research, clinical, industry, civil society) as well as for increasing HBV cure advocacy. A concerted effort to work together and facilitate cross-talk and exchange of knowledge and ideas will be vital.

It is important to acknowledge the context in which a cure is being developed. Relationships with potential clinical consequences such as hepatocellular carcinoma in HBV carriers need to be taken into consideration. Effective cure therapies will need to be measured against the ever-improving ARVs and an existing vaccine. A major barrier to finding an HBV cure is that research remains heavily underfunded. Despite progress in treatment and the availability of a prophylactic vaccine, increasing global mortality from chronic HBV infection, currently approximately at 800,000 individuals per annum, more than supports the belief that a cure is indeed necessary.

A cure for either HIV and(or) HBV infections is unlikely to be a magic bullet and will require a combination of approaches involving new therapies. Given the available technologies, we can explore human immunology with precision using small amounts of clinical

material. This opportunity should be leveraged. Clinical research ought to be shifted from the much studied white males to include groups such as natural and post-treatment controllers, and more women in cure trials as well as communities generally under-represented in clinical trials such as those of African descent in order to be able to assess efficacy in all populations. Given that many cure therapies will involve unknown risks, there will be obstacles in communicating the risks to study participants and community engagement will be key to cure development.

Identifying the adequate target populations for such a goal, addressing stigma, accessing hard-to-reach populations, ensuring drug and partner safety and obtaining fully-informed consent from participants are some of the key ethical considerations that are potential barriers to HIV-1 and HBV cures.

7 Closing session

Raymond Schinazi (Emory University, Atlanta, USA) gave the Forum's closing lecture on 'Disruptive discoveries for HBV treatment and eradication'.

Targeting the HBV capsid with small molecules called capsid assembly effectors (CAEs) is a promising strategy towards HBV elimination as the capsid is key to several steps of viral replication. These include packaging of pregenomic viral RNA, viral particle budding from the endoplasmic reticulum and maintaining the all-important cccDNA. The CAEs thus potentially disrupt many steps in HBV replication. More than 175 compounds have been synthesised and tested in various models of HBV replication. Lead compounds were selected from those that most effectively inhibited production of markers of HBV replication in cultured cells and led to the identification of GLP-26. Secretion of HBeAg, a surrogate marker for cccDNA function, was also reduced in cultured HBV-infected HepG2-NTCP cells. Moreover, a cccDNA decreased concentration confirmed that the compound had an effect on this replication intermediate. Evaluation *in vivo* showed that GLP-26 was bioavailable after oral administration with a long half-life. Good efficacy was also demonstrated using the model of HBV-infected immunocompromised mice that had been xenografted with human hepatocytes. Combination with entecavir (ETV) showed synergistic action with delayed viral rebound, ranging from 3 to 9 weeks, following cessation of treatment. These promising results have prompted clinical evaluation, which is anticipated during mid-2020.

An update from the Sunnylands Summit, 'The Path Towards ending HIV' was presented by Sharon Lewin (Doherty Institute, Melbourne, Australia). The meeting, jointly hosted by the IAS and the Annenberg Trust at Sunnylands, aimed to define a target product profile (TPP) for an HIV-1 cure applicable in low and lower-middle income settings, taking into account the expected technological, delivery and funding landscape of HIV-1 interventions, such as the future use of long-acting ART, bNAbs and various treatment modalities. Following this summit, two key outputs were determined: the creation of the HIV Cure Africa Acceleration Partnership (HCAAP), a public-private partnership focusing on low and lower-middle income countries in Africa and the establishment of a working group to develop TPPs for an HIV cure.

Acknowledgements

The authors would like to acknowledge the Forum co-chairs Steven Deeks, Anna Kramvis, Sharon Lewin, Seng Gee Lim and the Forum Programme Committee. The Forum is indebted to Rosanne Lamplough and Jessica Jexler, IAS for organising the meeting.

Funding

The 2019 HIV & HBV Cure Forum was supported by Gilead, ViiV Healthcare, the French National Agency for Research on AIDS and viral hepatitis (ANRS), MSD and the Mexican Social Security Institute (IMSS).

Conflicts of interest

P. Arbutnot, R. Rajasuriar, M. Paximadis and S. Perez Patrigeon have no conflicts of interest to declare. Eileen Scully has consulted for Merck.

References

- Königer C, Wingert I, Marsmann M *et al.* Involvement of the host DNA-repair enzyme TDP2 in formation of the covalently closed circular DNA persistence reservoir of hepatitis B viruses. *Proc Natl Acad Sci USA* 2014; **111**(40): E4244–E4253.
- Decorsière A, Mueller H, van Breugel PC *et al.* Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. *Nature* 2016; **531**(7594): 386–389.
- Lucifora J, Xia Y, Reisinger F *et al.* Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343**(6176): 1221–1228.
- Urban S, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. *Gastroenterology* 2014; **147**(1): 48–64.
- Cohn LB, Silva IT, Oliveira TY *et al.* HIV-1 integration landscape during latent and active infection. *Cell* 2015; **160**(3): 420–432.
- Bruner KM, Wang Z, Simonetti FR *et al.* A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 2019; **566**(7742): 120–125.
- Lorenzi JC, Cohen YZ, Cohn LB *et al.* Paired quantitative and qualitative assessment of the replication-competent HIV-1 reservoir and comparison with integrated proviral DNA. *Proc Natl Acad Sci USA* 2016; **113**(49): E7908–E7916.
- Gaebler C, Lorenzi JCC, Oliveira TY *et al.* Combination of quadruplex qPCR and next-generation sequencing for qualitative and quantitative analysis of the HIV-1 latent reservoir. *J Exp Med* 2019; **216**(10): 2253–2264.
- Chun TW, Finzi D, Margolick J *et al.* In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat Med* 1995; **1**(12): 1284–1290.
- Chun TW, Carruth L, Finzi D *et al.* Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997; **387**(6629): 183–188.
- Bui JK, Sobolewski MD, Keele BF *et al.* Proviruses with identical sequences comprise a large fraction of the replication-competent HIV reservoir. *PLoS Pathog* 2017; **13**(3): e1006283.
- Hosmane NN, Kwon KJ, Bruner KM *et al.* Proliferation of latently infected CD4. *J Exp Med* 2017; **214**(4): 959–972.
- Wang Z, Gurule EE, Brennan TP *et al.* Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane. *Proc Natl Acad Sci U S A* 2018; **115**(11): E2575–E2584.
- Pardons M, Baxter AE, Massanella M *et al.* Single-cell characterization and quantification of translation-competent viral reservoirs in treated and untreated HIV infection. *PLoS Pathog* 2019; **15**(2): e1007619.
- Cohn LB, da Silva IT, Valieris R *et al.* Clonal CD4⁺ cells inf the HIV-1 latent reservoir display a distinct gene profile upon reactivation. *Nat Med* 2018; **24**(5): 604–609.
- Gierahn TM, Wadsworth MH, Hughes TK *et al.* Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput. *Nat Methods* 2017; **14**(4): 395–398.
- Lu CL, Pai JA, Nogueira L *et al.* Relationship between intact HIV-1 proviruses in circulating CD4. *Proc Natl Acad Sci USA* 2018; **115**(48): E11341–E11348.
- Farber DL, Yudanin NA and Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* 2014; **14**(1): 24–35.
- Maldarelli F, Wu X, Su L *et al.* HIV latency. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. *Science* 2014; **345**(6193): 179–183.
- Pinzone MR, VanBelzen DJ, Weissman S *et al.* Longitudinal HIV sequencing reveals reservoir expression leading to decay which is obscured by clonal expansion. *Nat Commun* 2019; **10**(1): 728.
- Hiener B, Horsburgh BA, Eden JS *et al.* Identification of genetically intact HIV-1 proviruses in specific CD4. *Cell Rep* 2017; **21**(3): 813–822.
- Revill PA, Chisari FV, Block JM *et al.* A global scientific strategy to cure hepatitis B. *Lancet Gastroenterol Hepatol* 2019; **4**(7): 545–558.
- Hu J, Cheng J, Tang L *et al.* Virological basis for the cure of chronic hepatitis B. *ACS Infect Dis* 2019; **5**(5): 659–674.
- Coffin CS, Zhou K and Terrault NA. New and old biomarkers for diagnosis and management of chronic hepatitis B virus infection. *Gastroenterology* 2019; **156**(2): 355–368.e353.
- Block TM, Locarnini S, McMahon BJ *et al.* Use of current and new endpoints in the evaluation of experimental hepatitis B therapeutics. *Clin Infect Dis* 2017; **64**(9): 1283–1288.
- Riddell J, Amico KR and Mayer KH. HIV preexposure prophylaxis: a review. *JAMA* 2018; **319**(12): 1261–1268.
- Falkenhagen A and Joshi S. Genetic strategies for HIV treatment and prevention. *Mol Ther Nucleic Acids* 2018; **13**: 514–533.
- Hütter G. More on shift of HIV tropism in stem-cell transplantation with CCR5 delta32/delta32 mutation. *N Engl J Med* 2014; **371**(25): 2437–2438.
- Delville M, Touzot F, Couzin C *et al.* Safety of CD34. *Mol Ther Methods Clin Dev* 2019; **13**: 303–309.
- Tebas P, Stein D, Tang WW *et al.* Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N Engl J Med* 2014; **370**(10): 901–910.
- Dash PK, Kaminski R, Bella R *et al.* Sequential LASER ART and CRISPR treatments eliminate HIV-1 in a subset of infected humanized mice. *Nat Commun* 2019; **10**(1): 2753.

32. Maude SL, Frey N, Shaw PA *et al*. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014; **371**(16): 1507–1517.
33. Deeks SG, Wagner B, Anton PA *et al*. A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. *Mol Ther* 2002; **5**(6): 788–797.
34. Maldini CR, Ellis GI and Riley JL. CAR T cells for infection, autoimmunity and allotransplantation. *Nat Rev Immunol* 2018; **18**(10): 605–616.
35. Wooddell CI, Yuen MF, Chan HL *et al*. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. *Sci Transl Med* 2017; **9**(409): eaan0241.
36. Bloom K, Maepa MB, Ely A and Arbutnot P. Gene therapy for chronic HBV-can we eliminate cccDNA? *Genes* 2018; **9**(4): E207.
37. Wagner DL, Amini L, Wendering DJ *et al*. High prevalence of *Streptococcus pyogenes* Cas9-reactive T cells within the adult human population. *Nat Med* 2019; **25**(2): 242–248.
38. Charlesworth CT, Deshpande PS, Dever DP *et al*. Identification of preexisting adaptive immunity to Cas9 proteins in humans. *Nat Med* 2019; **25**(2): 249–254.
39. Bloom K, Ely A, Mussolino C *et al*. Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered transcription activator-like effector nucleases. *Mol Ther* 2013; **21**(10): 1889–1897.
40. Chen J, Zhang W, Lin J *et al*. An efficient antiviral strategy for targeting hepatitis B virus genome using transcription activator-like effector nucleases. *Mol Ther* 2014; **22**(2): 303–311.
41. Naran K, Nundalall T, Chetty S and Barth S. Principles of immunotherapy: implications for treatment strategies in cancer and infectious diseases. *Front Microbiol* 2018; **9**: 3158.
42. Shata MTM, Abdel-Hameed EA, Rouster SD *et al*. HBV and HIV/HBV infected patients have distinct immune exhaustion and apoptotic serum biomarker profiles. *Pathog Immun* 2019; **4**(1): 39–65.
43. Halper-Stromberg A and Nussenzweig MC. Towards HIV-1 remission: potential roles for broadly neutralizing antibodies. *J Clin Invest* 2016; **126**(2): 415–423.
44. Barouch DH, Whitney JB, Moldt B *et al*. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature* 2013; **503**(7475): 224–228.
45. Shingai M, Nishimura Y, Klein F *et al*. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature* 2013; **503**(7475): 277–280.
46. Bar KJ, Sneller MC, Harrison LJ *et al*. Effect of HIV antibody VRC01 on viral rebound after treatment interruption. *N Engl J Med* 2016; **375**(21): 2037–2050.
47. Crowell TA, Colby DJ, Pinyakorn S *et al*. Safety and efficacy of VRC01 broadly neutralising antibodies in adults with acutely treated HIV (RV397): a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet HIV* 2019; **6**(5): e297–e306.
48. Mendoza P, Gruell H, Nogueira L *et al*. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature* 2018; **561**(7724): 479–484.
49. Nishimura Y, Gautam R, Chun TW *et al*. Early antibody therapy can induce long-lasting immunity to SHIV. *Nature* 2017; **543**(7646): 559–563.
50. Mouquet H. Hunting down the HIV-1 reservoir: a starring role for antibodies? *Immunity* 2017; **46**(4): 527–529.
51. Borducchi EN, Liu J, Nkolola JP *et al*. Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature* 2018; **563**(7731): 360–364.
52. Wu X, Guo J, Niu M *et al*. Tandem bispecific neutralizing antibody eliminates HIV-1 infection in humanized mice. *J Clin Invest* 2018; **128**(6): 2239–2251.
53. Ferrari G. Tandem bispecific broadly neutralizing antibody - a novel approach to HIV-1 treatment. *J Clin Invest* 2018; **128**(6): 2189–2191.
54. Schneider JR, Carias AM, Bastian AR *et al*. Long-term direct visualization of passively transferred fluorophore-conjugated antibodies. *J Immunol Methods* 2017; **450**: 66–72.
55. Stephenson KE. Therapeutic vaccination for HIV: hopes and challenges. *Curr Opin HIV AIDS* 2018; **13**(5): 408–415.
56. Schurich A, Pallett LJ, Lubowiecki M *et al*. The third signal cytokine IL-12 rescues the anti-viral function of exhausted HBV-specific CD8 T cells. *PLoS Pathog* 2013; **9**(3): e1003208.
57. Bertoletti A and Ferrari C. Adaptive immunity in HBV infection. *J Hepatol* 2016; **64**(1 Suppl): S71–S83.
58. Lewandowska M and Piekarska A. New directions in hepatitis B therapy research. *Clin Exp Hepatol* 2017; **3**(3): 119–126.
59. Das A, Ellis G, Pallant C *et al*. IL-10-producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. *J Immunol* 2012; **189**(8): 3925–3935.
60. Burton AR, Pallett LJ, McCoy LE *et al*. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. *J Clin Invest* 2018; **128**(10): 4588–4603.
61. Boni C, Barili V, Acerbi G *et al*. HBV immune-therapy: from molecular mechanisms to clinical applications. *Int J Mol Sci* 2019; **20**(11): E2754.
62. Maini MK and Peppas D. NK cells: a double-edged sword in chronic hepatitis B virus infection. *Front Immunol* 2013; **4**: 57.
63. Fabozzi G, Pegu A, Koup RA and Petrovas C. Bispecific antibodies: potential immunotherapies for HIV treatment. *Methods* 2019; **154**: 118–124.
64. Ferrari G, Haynes BF, Koenig S *et al*. Envelope-specific antibodies and antibody-derived molecules for treating and curing HIV infection. *Nat Rev Drug Discov* 2016; **15**(12): 823–834.
65. Liu B, Zhang W and Zhang H. Development of CAR-T cells for long-term eradication and surveillance of HIV-1 reservoir. *Curr Opin Virol* 2019; **38**: 21–30.
66. Harper J, Adams KJ, Bossi G *et al*. An approved in vitro approach to preclinical safety and efficacy evaluation of engineered T cell receptor anti-CD3 bispecific (ImmTAC) molecules. *PLoS One* 2018; **13**(10): e0205491.
67. Liddy N, Bossi G, Adams KJ *et al*. Monoclonal TCR-redirection tumor cell killing. *Nat Med* 2012; **18**(6): 980–987.
68. Yang H, Buisson S, Bossi G *et al*. Elimination of latently HIV-infected cells from antiretroviral therapy-suppressed subjects by engineered immune-mobilizing T-cell receptors. *Mol Ther* 2016; **24**(11): 1913–1925.
69. Pace MJ, Graf EH, Agosto LM *et al*. Directly infected resting CD4+T cells can produce HIV Gag without spreading infection in a model of HIV latency. *PLoS Pathog* 2012; **8**(7): e1002818.
70. Cai Y, Abdel-Mohsen M, Tomescu C *et al*. BCL6 inhibitor-mediated downregulation of phosphorylated SAMHD1 and T cell activation are associated with decreased HIV infection and reactivation. *J Virol* 2019; **93**(2): e01073–18.
71. Cardenas MG, Yu W, Beguelin W *et al*. Rationally designed BCL6 inhibitors target activated B cell diffuse large B cell lymphoma. *J Clin Invest* 2016; **126**(9): 3351–3362.
72. Watson DC, Moysi E, Valentin A *et al*. Treatment with native heterodimeric IL-15 increases cytotoxic lymphocytes and reduces SHIV RNA in lymph nodes. *PLoS Pathog* 2018; **14**(2): e1006902.
73. Cohen JA, Barkhof F, Comi G *et al*. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med* 2010; **362**(5): 402–415.
74. Kappos L, Radue EW, O'Connor P *et al*. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med* 2010; **362**(5): 387–401.
75. Maini MK and Pallett LJ. Defective T-cell immunity in hepatitis B virus infection: why therapeutic vaccination needs a helping hand. *Lancet Gastroenterol Hepatol* 2018; **3**(3): 192–202.
76. Lim SY, Osuna CE, Hraber PT *et al*. TLR7 agonists induce transient viremia and reduce the viral reservoir in SIV-infected rhesus macaques on antiretroviral therapy. *Sci Transl Med* 2018; **10**(439): eaao4521.
77. Borducchi EN, Cabral C, Stephenson KE *et al*. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature* 2016; **540**(7632): 284–287.
78. Lopatin U, Wolfgang G, Tumas D *et al*. Safety, pharmacokinetics and pharmacodynamics of GS-9620, an oral Toll-like receptor 7 agonist. *Antivir Ther* 2013; **18**(3): 409–418.
79. Fisicaro P, Valdatta C, Massari M *et al*. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. *Gastroenterology* 2010; **138**(2): 682–693, 693.
80. Gane E, Verdon DJ, Brooks AE *et al*. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: a pilot study. *J Hepatol* 2019; S0168-8278(19)30400-3 (Epub ahead of print).
81. Ho YC, Shan L, Hosmane NN *et al*. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 2013; **155**(3): 540–551.