FULL RECOMMENDATIONS:
TOWARDS AN HIV CURE 2016
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This document is an extended Full Recommendations companion text to the Perspective article International AIDS Society global scientific strategy: towards an HIV cure 2016. Some of the text and the figures contained in the Full Recommendations were first published in ref. 30.
The HIV/AIDS pandemic represents the most important global health challenge in modern history. The tremendous advances in biomedical research, in particular effective antiretroviral treatment (ART), provided the means to control the virus, dramatically improving the health and life expectancy of people living with HIV.

Nevertheless, current treatment strategies still have a number of limitations. The immense operational and logistical challenges in delivering life-long care remain daunting. The sheer economic costs of providing effective ART to the 37 million people now living with HIV is prohibitive and unsustainable. Drug toxicities, persistent immune dysfunction and inflammation and excess risk of co-morbidities have important health consequences. Then there are the considerable psychological issues related to HIV infection. These factors all highlight the urgency of identifying an effective means to control the virus in the absence of therapy. The search for a curative strategy for HIV is therefore a goal of paramount importance, and the area is increasingly being defined as a key priority for the future of HIV research.

The definition of “cure” is important to clarify for researchers, clinicians and people living with HIV. The optimal outcome would be the complete eradication within an individual of all replication-competent HIV. Such a sterilizing cure will be challenging to achieve and will be impossible to prove with the current knowledge and technology. A more practical outcome will be the achievement of a long-term remission, generally defined as absence of viral rebound after ART cessation for an as-yet undefined period of at least several years. Remission, defined as the “lessening the severity of a disease or symptom for a period”, is likely a necessary precursor towards the development of an HIV cure, and is increasingly utilized in the field to indicate the goal of long-term undetectable viremia in the absence of ART. The concept of disease remission denotes improvement albeit with some uncertainty and is already well entrenched in medical settings.

The crux of the challenge lies in the ability of HIV to persist indefinitely even while an individual is on antiretroviral therapy. Indeed, the primary barrier to a cure is the existence of a stable reservoir of silenced fully integrated HIV DNA in long-lived cellular reservoirs. Most CD4+ T cells that are productively infected with HIV appear to die from virus-induced cytopathic effects or immune clearance. However, a small population of memory T cells that lack activation markers can be repeatedly isolated from blood despite durable, successful antiretroviral therapy, and are found to harbour integrated HIV DNA capable of producing replication-competent virus. Such infected cells appear to persist for years as the frequency of this population in blood changes little despite prolonged therapy. These cells are enriched in lymphoid tissues, particularly secondary lymph nodes, the spleen and mucosal surfaces. The rebound of viremia from these reservoirs upon interruption of ART represents the major challenge in achieving a cure for HIV.

Nevertheless, HIV cure research has made remarkable advances over the past four years, coming into its own not only as a scientific goal, but also as an expanding multidisciplinary field of inquiry. Encouraging results demonstrate that a cure and sustained HIV remission is possible in the absence of antiretroviral therapy (Figure 1), albeit so far in a very limited number of cases. Indeed, ART interruption is generally followed by rebound viremia within two to three weeks, with several notable exceptions. Timothy Ray Brown, often referred to as the Berlin Patient, received a stem cell transplant using donor stem cells that were homozygous for CCR5Δ32 and is considered to be the only person known to be effectively cured of HIV. However, a similar approach in two people from Boston living with HIV who received allogeneic stem cell transplants (without the CCR5 mutation) for cancer treatment was not successful in eradicating HIV; both patients showed viral rebound, albeit significantly delayed after ART interruption. These sobering results highlighted the difficulty in detecting persistent HIV.
Interestingly, several cases of sustained HIV remission, in which the virus remains present at extremely low levels but is seemingly controlled without ART, have been described in individuals treated very early after their infection by HIV. Combination ART initiated rapidly after HIV infection has been shown to limit the reservoir size and limit the negative impact on the immune system. In 2013, The report on the Visconti study (viro-immunological sustained control after treatment interruption) described a group of patients who had received early treatment and were able to control viremia in the absence of treatment, effectively achieving HIV remission, some for up to 10 years off therapy.53 This specific group of individuals does not display the characteristic genetic background of spontaneous HIV controllers, suggesting that other mechanisms enable viral control. In 2016, the same group presented the case of a teenager showing viral remission more than 12 years after ART interruption.6

The Mississippi Child also received very early ART, shortly after birth until 18 months of age,3 and then did not rebound for nearly 28 months.61 This strengthened the hypothesis that early treatment may subsequently lead to viremia control, although sometimes only temporarily. The mechanisms behind this control remain to be elucidated.

Although HIV cure research is a new part of HIV clinical research, the essential ethical principles that underpin sound clinical trials are the same.62-64 An ethical review committee should evaluate all HIV cure research prior to starting the research. Given the substantial participant risk associated with many HIV cure trials and the possibility of therapeutic misconception, a thorough informed consent process is essential.65 The key ethical challenges should be addressed not only in the research stage, but also in the implementation of any cure strategies.

The International AIDS Society (IAS) originally set up its initiative, entitled “Towards an HIV Cure”, to underline the aspiration of identifying a cure in line with a range of programmes from funding agencies and philanthropic organizations. A major component of this initiative was the development of a long-term scientific strategy by a large, multidisciplinary group of scientists (the Global Scientific Strategy: Towards an HIV Cure). This report, released in
2012, highlighted the areas and gaps that had to be addressed to accelerate research towards a cure for HIV. Given the highly dynamic nature of HIV cure research, the initiative reconvened a new panel, adding new members with unique expertise relevant to the emerging agenda. This second edition of the Global Scientific Strategy: Towards an HIV Cure presents the recommendations of an International Scientific Working Group on the areas and gaps that must be addressed to accelerate research towards a cure for HIV.

**TEXT BOX 1: DEFINING “CURE” IN HIV DISEASE**

The definition of “cure” is important to clarify for researchers, clinicians and people living with HIV. The optimal outcome would be the complete eradication within an individual of all replication-competent HIV. Such a sterilizing cure will be challenging to achieve and will be impossible to prove with the current technologies. A more feasible outcome will be the achievement of a long-term remission. Remission is likely to be a necessary precursor towards the development of an HIV cure and is increasingly utilized in the field to indicate the goal of long-term undetectable viremia for an as-yet undefined period (likely of several years) in the absence of ART. The concept of disease remission denotes improvement albeit with some uncertainty and is already well entrenched in medical settings.

**TEXT BOX 2: PROGRESS IN HIV CURE RESEARCH 2012-2016**

There have been a number of significant advances since the publication of the first 2012 Global Scientific Strategy: Towards an HIV Cure.

Sustained periods of aviremia in the absence of therapy was achieved in an aggressively treated infant, in at least two individuals who have received allogeneic stem cell transplant, and in adults who received several years of ART initiated soon after infection (Figure 1). Non-human primate and humanized mouse models of well-treated SIV/HIV disease have been validated and used to advance the scientific agenda. Most HIV in blood was found to be replication-incompetent and most of the apparent replication-competent virus was found to be non-inducible ex vivo. Early initiation of ART limits the establishment of the reservoir and prevents the generation of immune escape in latently infected cells.

New tools that can quantify the frequency of a cell that carries replication-competent virus have been developed, and some biomarkers have been shown to predict the time to viral rebound following a treatment interruption. The central role of the T follicular helper cells and the B cell follicle in supporting SIV/HIV replication was established. The central role of long-lived self-renewing memory CD4+ T cells as a reservoir during sustained ART (>10 years) was established, while the role of monocytes/macrophages as a stable reservoir during ART was challenged. Homeostatic proliferation induced by cytokines or HIV integration events as a mechanism of persistence was demonstrated.

Evidence was presented suggesting that HIV continues to replicate and evolve during the first six months of ART but not necessarily during long-term ART. New latency reversing agents and combination approaches were identified in vitro and the capacity of more established “latency-reversing agents” (LRAs) to disrupt latency was demonstrated in a series of Phase I/II clinical trials. Novel vaccines were developed that contained and possibly cured SIV infection when administered before infection, and the safety and potential efficacy of broadly neutralizing antibodies and bi-specific antibodies demonstrated. Gene therapy with CCR5 modification was found to be feasible and safe.
MOLECULAR BIOLOGY OF HIV LATENCY AND REVERSAL STRATEGIES

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1. BACKGROUND

Latency is defined as the persistence of integrated viral DNA that is replication competent but transcriptionally silent. Although most infected cells, and therefore the majority of persistently infected cells, are found in tissues, HIV latency has primarily been studied in laboratory models or in cells obtained from the circulation. Latency was originally described in resting memory T cells, but has been described in other T cell populations, such as naive CD4+ T cells and stem central memory T cells, as well as tissue macrophages, astrocytes and thymocytes. The establishment of latency in resting memory T cells is due either to the infection of resting CD4+ T cells (which is difficult to achieve ex vivo in the absence of a second non-activating stimulus) or to the infection of highly susceptible activated CD4+ T cells followed by their reversion to a resting state (Figure 2). A recent study suggested that infection in vitro within tissues may establish quiescence at a higher frequency.

The transcription of HIV DNA can be divided in two phases. The first is driven by the transcription machinery of the host genome. The second requires the coordinated action of the HIV-encoded regulatory protein Tat and cellular transcription machinery. In vitro studies using cellular models that recapitulate HIV transcriptional latency suggest several mechanisms for transcriptional repression of the viral promoter.

First, there is low expression or lack of recruitment of general transcription factors required for different steps of the viral transcription. This includes the TATAA binding protein (TBP) required for transcription initiation, transcription factor IIH (TFIIH) needed for promoter clearance, and/or the positive transcription elongation factor b (p-TEFB) that allows transcription elongation and RNA processing. As a consequence, negative transcriptional regulators (such as nuclear factor, NFκB p50 homodimers) are recruited to the viral long terminal repeat (LTR), and a repressive chromatin environment is established through the recruitment of histone deacetylases and histone methyltransferases and/or negative transcription elongation factor (NTEF) to impose a block to transcription elongation. On the other hand, insufficient levels or lack of recruitment of host transcription factors – such as nuclear factor κB p50/p65, nuclear factor of activated T cells (NFAT), signal transducer and activator of transcription 5 (STAT5) and p-TEFB – may result in the inability to overcome both chromatin repression and transcription elongation block.

More recently, additional factors that may directly restrict the process of transcription at the HIV promoter, and insufficient cellular levels of other key factors, such as cyclins, have been reported to play key roles in maintaining latency within quiescent cells. Further, transcriptional interference by host promoter activities has been reported to block viral gene expression. While most studies have focused on viral and host proteins governing latency, new evidence suggest that miRNAs might also function as pro-latency factor given their propensity to promote inhibition of HIV replication. Finally, it has become clear that the process of transcriptional activation within a population of proviral genomes is not an all-or-nothing process, and that the multiple levels of transcriptional control confer a stochastic nature to proviral expression whereby even in the face of a maximal cascade of activating signals, latency cannot be disrupted in an entire population of proviral genomes by a singular event.
FIGURE 2. MECHANISMS THAT MAINTAIN HIV LATENCY IN RESTING CD4+ T CELLS. There are multiple blocks to viral production in latently infected resting CD4+ T cells. TCR = T cell receptor; TF = transcription factors; CoAct = co-activators; MS = multiply spliced; US = unspliced; miRNA = microRNA

In addition to these molecular mechanisms acting in the nucleus, persistently infected cells may be maintained by factors that result in an extended lifespan and/or homeostatic proliferation of the cells.\textsuperscript{57,86} Of note, the host pathways that enable lifelong immunological memory also result in lifelong infection; hence, it is expected that advances in our understanding of latency will be informed by and contribute to our understanding of the basic immunology of T cell memory. Therapies that promote the expression of viral proteins from persistently infected cells by modifying these pathways have been or are currently being tested.

One of the most studied strategies for the eradication of the HIV reservoir has focused on purging reservoirs using anti-latency agents, such as HDAC inhibitors, while preventing renewed infection by continuous ART. However, several other strategies have been proposed or studied, including ART regimen intensification, vaccination, gene therapy, stem cell transplantation\textsuperscript{87} or the use of therapeutic agents targeting HIV transcription to drive viral genomes into long-term, deep latency that might be refractory to viral reactivation.\textsuperscript{88,89}
As discussed above, a panoply of cellular mechanisms have been defined that contribute to the establishment and maintenance of persistent proviral infection. These mechanisms have largely been discovered and studied in cell line models or primary cell models of resting central memory CD4+ T cells and, in a few cases, validated in primary cell populations of HIV-infected individuals on ART. However, with regards to clinical translational research towards an HIV cure, a number of critical gaps remain. First, as will be discussed in detail later, while the pool of central memory CD4+ T cells has been well studied, evidence of HIV latency within other CD4+ T cell populations and within other non-T cell populations has been described. The frequency and, of greatest importance, the durability of persistent infection within these other cell populations requires further definition and is a major gap in our knowledge.

Second, singular blocks to the expression of persistent provirus, such as the deacetylation of nucleosomes about the HIV LTR, have been successfully targeted in several translational experiments, providing some preliminary proof of concept for this general approach. However, while histone deacetylation is clearly associated with latency disruption, it cannot yet be formally shown that this molecular event is either necessary or sufficient for proviral reinduction. And despite the evidence for induction of proviral transcriptional in these clinical experiments, there has been no effect on reservoir size, and so the efficacy of latency reversal as a stand-alone approach appears limited. We lack an understanding of whether this apparent lack of efficacy is due to an absolute requirement to disrupt multiple restrictions to proviral expression, to an insufficient duration or potency of the approaches used thus far, to the inability of patients durably treated with antiretrovirals to recognize and clear cells in which latency has reversed, or to some combination of these shortcomings.

Further, we lack validated systems with which to test and compare different “latency-reversing agents” (LRAs) and, of critical importance, we lack systems in which to study these effects over time. Due to the stochastic nature of proviral expression, it appears most likely that effective disruption of latency will require repeated administration of LRAs to fully extinguish the population of cells capable of giving rise to viral rebound in the absence of continued antiretroviral therapy.

It remains unclear whether or not proviral latency can be fully reversed in a population of cells within an infected host without fundamentally altering the nature of resting, non-cycling immune cells. While it is clear that LRAs can act on a fraction of persistently infected cells, fully effective anti-latency therapy may require the redistribution or upregulation of several key cellular factors that regulate gene transcription within the immune system. Studies of these critical gaps will also require the use of representative in vivo model systems as resting cells studied in culture may represent an artificially unresponsive system, devoid of the cellular interactions and signalling found in vivo.

Finally, therapeutic strategies exploring suppression of proviral latency rather than activation of the persistently infected cells will require validation in animal model systems. Transcriptional inhibitors, such as inhibitors of Tat, p-TEFb, NF-κB or Hsp90, with or without ART, could hypothetically reduce the size of the reservoir by blocking ongoing viral replication, reactivation and replenishment despite the persistence of the HIV genetic material in the body. HIV encodes for two regulatory proteins, Tat and Rev, that are required for viral transcription and viral genomic RNA and single spliced mRNA nuclear export, respectively. These two regulatory proteins function through unique mechanisms of action that, so far, are not shared by any of their cellular equivalents. Thus, based on our knowledge of how Tat and Rev achieve their function, together with available structural analyses, effort towards developing drugs able to inhibit Tat and Rev should be intensified. We still lack an understanding of the provirus following such “pharmacologically enforced” or “deep” latency and the durability of this state.
3. WHAT CHALLENGES HAVE TO BE OVERCOME TO ADVANCE IN THIS AREA?

The ultimate challenge is to determine the molecular mechanisms responsible for persistence of replication-competent proviral genomes. Achieving this goal has and is still facing a major obstacle due to the fact that resting CD4+ T cells carrying persistent provirus are rare and undistinguishable phenotypically from non-infected cells. The development of a cellular model based on resting CD4+ T cells is an absolute requirement for the achievement of this objective.

Such a model should reiterate the biology that underlies this process and the critical gaps in our understanding of proviral persistence despite successful antiretroviral therapy. Basic research on the cellular mechanisms that constitute the rate-limiting steps controlling gene expression in quiescent CD4+ T cells may help. Indeed, a better understanding of factors that allow or restrict transcriptional initiation and transcription complex processivity at the HIV LTR, and how to manipulate these factors therapeutically, is needed.

However, HIV RNA transcription is not the only event that is required to effectively disrupt latent infection and allow for targeting and clearance of persistently infected cells. HIV mRNA export and processing, and viral antigen expression, and/or processing and presentation has been relatively little studied of late, especially within resting memory T cell populations and other potential cell populations that define the reservoir. A full understanding of the steps and processes that allow a rare persistently infected cell to be revealed to the immune system, or to be targeted, is still lacking.

The low frequency of persistent infection in primary cells is another major challenge. However, while latency in central memory CD4+ T cells from patients is rare, the identification and study of other potential reservoirs has been even more challenging.

Finally, as progress is made towards the understanding of latency and means to reverse it, an additional challenge will be to develop effective means of augmenting the clearance of these rare infected cells. Formerly persistently infected T cells might only be induced to express low levels of antigen, or transiently, and in patients in whom immune dysfunction or exhaustion has occurred, in whom prolonged suppression of viremia has dampened the HIV-specific immune response, or where archived viral quasispecies express viral antigens that have evaded the adaptive immune response. It is likely that techniques to augment the adaptive or innate antiviral immune response will be needed, or that an engineered antiviral response will have to be administered. Therefore, as effective LRAs that target host cellular pathways are developed, these reagents will have to be carefully vetted for their potential to dampen the very immune responses needed for viral clearance.

4. RECOMMENDATIONS FOR FUTURE RESEARCH

The current most-studied paradigm for clearance or durable remission of HIV infection is based on the hypothesis that proviral latency can be interrupted, revealing persistent infection that can then be cleared. An alternative approach that represents a significant departure from established paradigms of eradicating the HIV reservoir involves the use of therapeutic agents targeting HIV transcription. These would suppress residual levels of viral transcription in persistently infected cells, thereby establishing a state of deep-latency refractory to viral reactivation. This strategy requires a greater degree of specificity with respect to the HIV promoter.
To tackle HIV persistence, major needs for future research include:

- A better understanding of the factors that restrict proviral expression in primary CD4+ T cells and other potential cellular reservoirs of persistent infection, and new tools and approaches to inhibit or target these restrictions. Restriction includes not just those that inhibit viral transcription, but also all steps of RNA translation and antigen production in cellular reservoirs.

- A better understanding of factors that are critically deficient in primary CD4+ T cells and other potential cellular reservoirs, and those that must be upregulated to allow viral transcription, RNA translation and antigen production. Research focused on deriving a minimal set of such factors required for latency reversal is needed as the likelihood is considerable that such perturbations of cellular state might result in immune activation, dysfunction or toxicity.

- A better knowledge of the details of transcriptional regulation of HIV compared with that of cellular genes. To our knowledge, two major properties have been already identified. First, HIV encodes for its transactivator Tat, which activates the HIV promoter by targeting TAR RNA. Thus, it might be possible to develop Tat-specific inhibitors without affecting general transcription of the host. The development of such inhibitors should take advantage of the fact that the crystal structure of Tat bound to P-TEFb has been reported. Second, the HIV promoter is highly sensitive to transcription elongation inhibitors compared with cellular genes. Whether this offers an acceptable safety margin for therapeutic intervention merits further investigation. If a limited exposure to such an inhibitor is sufficiently potent and durable, enforcement of latency could remove these cells as a potential source of viral rebound for a clinically relevant period of time (i.e., years).

### MOLECULAR BIOLOGY OF HIV LATENCY AND REVERSAL STRATEGIES:

#### PRIORITIES

- Continue development and refinement of resting CD4+ T cell models that reflect the diversity of proviral latency, to decipher the molecular mechanism of HIV latency, and are sufficiently tractable for use in therapeutic testing and development.

- Apply new tools, including single cell analyses to explore the diversity of proviral latency, and identify pathways and factors that can be potentially targeted in therapeutic testing and development.

- Explore the contribution of the HIV restriction factors in controlling the establishment and the maintenance of HIV latency.

- Focus on assay systems in patient cells to assess a range of responses: enforcement of latency, RNA expression, antigen expression and virion production.
VIRAL RESERVOIRS, IMMUNOLOGY
OF HIV PERSISTENCE AND
“KILL” STRATEGIES

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1. BACKGROUND

HIV causes profound and irreversible harm to the immune system. Within days of infection, much of the CD4+ T cell population in the gastrointestinal tract is depleted, resulting in loss of mucosal integrity and systemic exposure to gut microbial products.92 HIV replication over time leads to progressive loss of CD4+ T cells systemically, and a chronic inflammatory state emerges. Effective ART reverses many of these abnormalities, but a state of persistent inflammation and immune dysfunction typically persists. This immune state during ART is characterized by chronic low-level inflammation within the adaptive and innate immune systems,93,94 elevated immunoregulatory responses, CD4+ and CD8+ T cell dysfunction, and lymphoid fibrosis.95 It is expected that this compromised immune state contributes to HIV persistence and that efforts to clear the reservoir may require that some or all of these immunologic abnormalities be reversed.30,96

Most curative interventions being actively pursued involve some combination of latency reversal and clearance of virus-producing cells (“shock and kill”).35,36 The most effective means to reverse latency in vitro (the “shock”) is to activate CD4+ T cells in a potent and non-specific manner.34 Activation of CD4+ T cells in vivo may thus be necessary to reverse latency, although such efforts will have to be pursued conservatively given potential for adverse effects. The most effective means to clear virus-producing cells (the “kill”) is likely to involve manipulation of natural host immune-clearance mechanisms – mechanisms that generally fail profoundly after the post-acute phase of infection. Immune-based therapeutics that are informed by the immunology of HIV persistence will likely be a major focus of cure research in the future.96

Not all HIV that persists in vivo is replication competent and not all virus that can replicate is readily inducible ex vivo.9,97 All virus should be regarded as potentially inducible to varying degrees – from producing HIV proteins alone through to mature infectious virions. Although the reservoir of truly infectious virus should be the target of assay and drug development, much may be learned concerning host mechanisms of virus inactivation by studying infected cells that do not produce infectious virus.

2. WHAT ARE THE GAPS IN OUR UNDERSTANDING?

Persistently infected memory T cells in individuals on ART were identified by three independent laboratories in the mid-1990s55,56,98 Latency is established very early in the natural history of HIV infection,7,11,99 well before antiretroviral therapy can be administered. Despite nearly two decades of intense investigation, many fundamental aspects of the reservoir remain undefined. These deficits in the knowledge base fall into four broad categories: (1) nature of the cellular reservoir in terms of cell phenotype and tissue distribution; (2) mechanisms for persistence within these cellular and tissue reservoirs; (3) barriers to latency reversal; and (4) barriers to host-mediated clearance of infected cells.

There is no debate regarding the role of memory CD4+ T cells as a reservoir. In blood and tissues, it appears that the vast majority of the reservoir (defined in many ways) is in memory cells. In blood, most of the persistent virus is in central and transitional cells, while in the mucosa, most of the virus resides in effector memory cells (these differences largely reflect the abundance of these cell types in each compartment).57,100,101 Recently, a population of memory cells with self-renewing capacity was defined. These CD4+ memory stem cells are rare but very stable. It has been reported that over time, the reservoir becomes enriched in these cells,102 but much work needs to be done.

Many questions remain unanswered. Is HIV enriched in cells with certain antigen-specificity (e.g., HIV) during ART as it is in untreated disease? Can HIV be readily found in all CD4+ T cell populations, including T regulatory cells, Th1, Th2, Th17, and T follicular helper (Tfh) cells? The latter cell population is of high interest103 as it resides in B cell
Follicles of lymph nodes, and may represent a sanctuary for infected cells.\textsuperscript{18} Does the distribution within these cells change over time? One might assume that cells that are more long lived (e.g., resting central memory cells), that have self-renewing capacity (e.g., memory stem cells), that are protected from CTL (e.g., Th\textsubscript{f} cells), that are likely to remain in a quiescent state and that are more likely to become infected (e.g., activated cells in HIV-rich regions) will over time become enriched for HIV. Do myeloid cells harbour replication-competent HIV during long-term ART? Although infection in untreated disease is well established, it has proven difficult to demonstrate conclusively that replication-competent HIV persists indefinitely in these cells during ART\textsuperscript{22} Is the nature of the virus (e.g., inducible, non-inducible but replication competent and defective) the same in all cells? It can be safely assumed that rapid changes in the nature of the immune system during early life will dramatically affect the distribution of the virus in those who were infected before or at birth; early versus delayed ART in this understudied population would hence likely have dramatic effects on the nature of the reservoir. There is a compelling need for more specificity as it pertains to the nature of reservoir. Clonal expansion of HIV in memory CD4\textsuperscript{+} T cells is apparently common, perhaps because HIV integration alters how cell proliferation is regulated.\textsuperscript{26,27,104,105} It has been argued that HIV is enriched in cells that express markers of T cell activation and function, including HLA-DR, CCR5 and PD-1.\textsuperscript{57,106,107} Whether persistent virus in activated cells differs from resting cells remains unclear. The identification of cell-surface markers for the infected population could allow more targeted therapies. There is much to be learned regarding the distribution of the virus in different people. Does the nature of the infected memory cell population differ in those who are started on ART early versus late? What effect does age, gender, viral subtype and co-infections have on the size and distribution of the reservoir? Most studies have focused on largely Caucasian middle-aged and older men in resource-rich regions. Studies of other populations are urgently needed. It is generally accepted that the biology of CD4\textsuperscript{+} T cell memory determines the fate of persistently infected cells. Major knowledge gaps exist in this area. We do not know, for example, how latency is actually established. Were these cells infected during a state of activation with latency a natural consequence of a cell returning to rest, or are resting cells directly infected? We do know, however, that CD4\textsuperscript{+} T cells may persist for a very long time, potentially the lifespan of an individual. The question is whether they are actively turning over and what the fates of the daughter cells are. Does cytokine-mediated T cell turnover have distinct effects on latency compared with antigen-driven turnover? Do all memory subsets freely circulate? If not, what is the nature of the tissue-resident CD4\textsuperscript{+} T cells that harbour persistent HIV? Given that most CD4\textsuperscript{+} T cells reside in lymphoid tissues, what role do neighbouring cells have on persistent infection? Does signalling between antigen-presenting cells (e.g., dendritic cells, macrophages) and neighbouring infected cells contribute to latency?\textsuperscript{108} With regard to eliminating the reservoir, there are again many questions. Is virus inherently cytopathic? Will latency reversal lead in some or all cases to virus-mediated destruction of the infected cells? Is this different in resting and activated T cells? Are certain viral proteins more likely to be expressed in resting and activated cells? Once latency is reversed, which host mechanisms would be most likely to clear these cells? Are HIV-specific CD8\textsuperscript{+} T cells potentially active against the reservoir, or is there too much dysfunction and/or too much CTL escape to preclude therapeutic approaches aimed at these cells?\textsuperscript{10} Can HIV antibodies be re-engineered to target the reservoir? Which effector pathways should one target with these antibodies (e.g., natural killer cells, macrophages, complement)?\textsuperscript{109} Will the inflammatory or T cell-activating nature of latency reversal invariably lead to more cell proliferation, which could result in increased size of the reservoir?
The size of the reservoir has been linked to the level of T cell activation (defined variably). Can immune-based therapeutics that affect T cell activation and/or T cell proliferation contribute to a cure? Will the chronic inflammatory nature of the immune system during ART prevent CTL and other mechanisms from performing their normal functions, as has been argued to be the case in other chronic diseases, including cancer?

3. WHAT CHALLENGES HAVE TO BE OVERCOME TO ADVANCE IN THIS AREA?

There are a number of barriers that limit progress in addressing the aforementioned knowledge gaps.

The lack of a high throughput manner in which to sample, measure and characterize the total body burden of replication-competent HIV is likely the most important barrier preventing progress in the HIV cure arena; this challenge is addressed extensively in Chapter 5. Another pressing problem is the inability to fully study the reservoir in the most important tissues, at least in humans. There has been some success in accessing gut mucosa and peripheral lymph nodes, but such studies are challenging to perform in most centres and will likely never be possible to incorporate within large clinical trials.

There is very little known about HIV in very important tissue sites, including the central nervous system (CNS), the reproductive system and the spleen. These tissues are now being studied in animal models, but these models have limitations (e.g., less effective ART, shorter duration of ART, use of animal-specific viruses, such as SIV). Non-invasive imaging of the reservoir using radiolabeled SIV/HIV-specific antibodies or tracers has shown promise in animal models, but this approach fails to detect persistently infected cells.

The optimal manner in which to define how a pathway influences the virus population during ART is to therapeutically interrupt or to enhance that pathway in a controlled study. Many pathways of interest can be modified therapeutically, but there are many challenges in implementing studies of the pertinent drugs. Many (e.g., immune checkpoint blockers) were developed for cancer, and there is widespread hesitancy to use these drugs in chronic infectious disease settings for commercial reasons. Many of these approaches also have significant risks, which makes studies in generally healthy HIV-infected adults on ART challenging from an ethical perspective.

More interactions between HIV specialists and other disciplines working with these approaches are urgently needed but difficult to facilitate. There may be particular synergies between HIV and those working in oncology, rheumatologic diseases, aging and transplantation. Indeed, careful studies of HIV-infected adults undergoing transplantation have revealed novel insights regarding the reservoir and potential role of immune-modifying therapies. Careful studies of HIV-infected adults with cancer receiving immune checkpoint blockers and other emerging immunotherapies should prove to be particularly informative and may lead to identification of novel curative strategies for HIV infection.
Defining the reservoir

It is first and foremost important that we better define and quantitate the anatomical and cellular sites of HIV in the virologically suppressed individual, and assess their evolution over time. Without this information, we cannot know if a given “curative” strategy has made a meaningful impact. For accessible tissue sites (e.g., lymph node and gut), biopsies should be performed as in a number of current studies. Analyses should be performed on both flow-sorted cellular target cells and on tissue sections (e.g., by immunohistochemistry and in situ hybridization). For tissue sites that are less accessible (e.g., spleen, brain, genital and thymus), tissue banks should be accessed as these contain samples from both autopsies and biopsies of HIV-infected people. In addition, an effort should be made to establish new tissue banks to store samples that may be obtained at the time of elective surgery and stored under optimal conditions.

Emphasis should be placed on exploring how human variability (e.g., age, gender, co-morbidities, HIV disease progression state) affects the HIV reservoir. Since the majority of those infected with HIV around the world are chronically co-infected with such agents as malaria, tuberculosis and helminthic worms, the impact of such co-infections on the persistence of HIV should also be studied.

Emphasis should also be placed on the development and use of new technologies, such as single cell analysis ex vivo and non-invasive visualization of HIV reservoirs in vivo. Since the majority of those infected with HIV around the world are chronically co-infected with such agents as malaria, Mycobacterium tuberculosis and helminthic worms, the impact of such co-infections on the persistence of HIV should also be studied. We believe that similar studies in the SIV/Rhesus Macaque model would be useful for comparison and that findings in this model should be carefully cross-validated for relevance in humans. The humanized mouse models of HIV infection are also promising, but they have not been as well validated as the macaque model.

Defining the impact of ART on reservoir

More studies are needed regarding how the time of ART initiation affects both: (1) the size and distribution of the reservoir, and (2) long-term immune function. It is well established that the earlier ART is initiated, the lower the frequency of persistently infected cells. It is unknown, however, whether the timing of ART impacts HIV persistence in terms of its replication competence, its inducibility, its capacity to be reversed by latency-reversing agents and its capacity to be eliminated or controlled by immune-clearing mechanisms (e.g., CTL and antibodies). Similarly, although it is well established that early ART preserves immune function, it remains largely unknown whether important defects persist (particularly in tissues).

As the epidemic matures, more people will have been on suppressive ART for up to two decades. The true long-term stability of the reservoir in terms of size, distribution, inducibility and overall replication competence should be defined.

Defining the biology of the reservoir

We propose that studies regarding the basic biology of memory CD4+ T cell dynamics in antiretroviral-treated HIV/SIV-infected macaques and humans be carried out, focusing on cell populations known to be enriched for HIV or (when possible) cells harbouring persistent HIV. How CD4+ T cell memory is established and maintained in humans has not been fully characterized. The role of cells with stem cell-like properties is of particular interest.
We also propose that the relationship between the biology of memory T cells and latent infection be defined. This may involve transcriptomic and proteomic analysis of infected cells, as well as analysis of the transcriptional state on the virus.

There should be emphasis on how certain factors affect biology of T cell and non-T cell targets of HIV, and the subsequent effects on the virus. These factors should include but are not limited to: anatomical site, microenvironment, cellular metabolism, microbiome, co-morbidities and diet.

The most definitive manner in which to define the mechanisms for persistence in vivo is to interrupt or enhance discrete pathways in a controlled manner. Small, pathogenesis-driven proof-of-biology studies in macaques and humans will likely be necessary to identify why latency persists indefinitely and how it might be reversed or eliminated. Such studies are resource intensive. Such studies also require strong investments from industry.

We propose that a major effort should be expended in performing phase I/IIa safety and proof-of-biology/concept interventional studies coupled with state-of-the-art measurement of HIV reservoir. Given that the causes for reservoir establishment and persistence are likely multifactorial, we propose that combining therapeutic approaches in such early-phase studies are important. Many potential interventions pose substantial risk. In these cases, rigorous assessment of the interventions (safety, scientific rationale) may have to be first done in the non-human primate model.

The types of interventions proposed may be divided into two broad categories: (1) anti-proliferative/activation approaches that aim to reduce proliferation and activation of target cells, thereby reducing infection, virion production and the proliferation of integrated provirus; and (2) immune-modifying approaches that target one or more pathway and, as a result, have complex effects on inflammation, proliferation (homeostatic or other), immune exhaustion or response to cytokine or antigen-mediated activation.

For example, blocking PD-1 or the PD-1 ligand (PDL1) may restore immune function and response to viral antigens. PD-1 may also be downregulated by blocking signalling pathways, such as JAK/STAT, and JAK/STAT inhibitors may have the added benefit of reducing CD4+ T cell homeostatic proliferation and directly reduce the size of the persistent reservoir. Improving responses to HIV antigens through preserving memory CD8+ T cell function may also be a result of blocking various pathways, such as mTOR. However, these and other anti-inflammatory agents may inhibit effector T cell or NK cell function or increasing T regulatory (Treg) cell frequency or function, thereby hindering the clearance of reactivated cells in a combined “shock and kill” approach. The early-phase clinical study of the direct effects of immune-modifying agents on the persistent HIV reservoir alone or in combination with “shock” strategies are therefore critical to understanding what component or components of the immune responses will have to be harnessed or enhanced to achieve HIV eradication or long-term ART-free remission.

Activating T cells to reverse latency

It is generally accepted that the most efficient manner to reverse latency in vitro is via potent and broad activation of CD4+ T cells. Such interventions will likely prove too toxic to move into clinical trials. Interventions that more specifically target those pathways involved in maintaining latency, or that target those cells that are more likely to harbour HIV, might prove to be both effective and safe.

As an example, there are at least 10 distinct toll-like receptors (TLRs) in humans. These receptors recognize pathogen-associated or danger-associated molecular patterns. The TLRs are widely expressed on different cell types and stimulate distinct immunologic responses. Because the TLR pathways ultimately promote activation of CD4+ T cells in tissues, we propose that synergistic combinations of TLR agonists merit careful study as potentially potent latency reversing agents. Also, because activating TLR pathways stimulates powerful adaptive and innate (particularly NK cell) responses, they may enhance the capacity of the host to clear virus-producing cells.
A number of other potentially modifiable and immune-activating pathways exist and should be evaluated in vitro, in animal models and ultimately in humans.

Enhancing capacity of the immune system to clear or control HIV

HIV-antigen-directed interventions are aimed at targeting infected cells for killing either specifically (e.g., T cells, bNABs, bispecific antibodies vaccines) or non-specifically (e.g., NK cells). Clearly, there is overlap between these approaches as killing of infected cells with antibodies may necessitate antibody-dependent cellular cytotoxicity (ADCC) through natural killer (NK) cells and macrophages. It is also clear that some kind of intervention to make infected cells “visible” to these immune interventions is a prerequisite for these approaches.

With regard to enhancing T cell function, a number of approaches should be pursued. T cell vaccines that are able to enhance HIV-specific immunity remain a priority. There is specific interest in approaches that stimulate responses against novel, non-dominant epitopes, given that CTL escape to standard (canonical) epitopes likely exist in the majority of individuals and that most vaccines appear to stimulate pre-existing memory responses. A potent cytomegalovirus (CMV) vector reengineered to stimulate sustained responses to novel, non-immunodominant epitopes has shown promise in animal models.

There is also intense interest in developing the recently characterized family of broadly neutralizing antibodies (bNABs) as potential curative interventions. The degree to which these antibodies target HIV proteins during latency reversal remains to be defined. The degree to which they are able to overcome the sequence diversity that emerges in chronic infection is also largely unknown. The effector mechanisms by which these antibodies might control or deplete the reservoir are not known. The degree to which therapies will have to target effector cells known to populate those regions within the lymphoid system that harbours much of the virus population should also be determined. Given intense interest in developing biologics for other disease, it is expected that advances in enhancing the pharmacology of monoclonal antibodies in general will have a huge impact on the development of these approaches.

Adjunctive therapies that reverse T cell and B cell dysfunction (e.g., inhibitors of the immune checkpoint blockers) and chronic upregulation of immunoregulatory signals may also be necessary. Indeed, there is an immunotherapy revolution going on within oncology. Drugs that reverse inflammation directly or reverse the negative effects of chronic inflammation on adaptive immunity are leading to major advances in management of a number of cancers.

Developing and maintaining well-characterized cohorts

The goal of HIV cure research is to develop an intervention that results in sustained control of HIV replication in the absence of any ongoing therapy (“remission”). Studies of those who control HIV naturally (“elite controllers”) have provided the strongest evidence to date that HIV-specific CD8+ T cell immunity can contribute to virus control. Most therapeutic vaccines are based on these findings.

More recently, a group of individuals who might have been destined for poorly controlled HIV was possibly turned into long-term controllers by the introduction of early ART. The development of larger cohorts of these “post-treatment controllers” will be needed to confirm these provocative findings. Such cohorts might prove valuable in identifying novel mechanisms of virus control. Other cohorts that will likely prove valuable include those of long-term antiretroviral-treated adults and children, as well as cohorts of individuals undergoing solid organ transplantation or of individuals receiving immunotherapy and other treatments for the management of cancer and other chronic disease.
Combination approaches

There is considerable interplay between the two categories of intervention. For example, anti-proliferative cytokines may have a detrimental effect on immune-based therapies while cytokines produced by immune cells may affect anti-proliferative approaches. It is important to state that it will be difficult to predict the outcome of such interventions in humans. For example, while type I interferon is pro-inflammatory and may be considered a promoter of HIV disease progression, its antiviral effects may be more beneficial in particular circumstances.

Finally, many potential modifiers of therapeutic interventions should be considered. These include host genetics, microbiome and co-morbidities. Indeed, some of these modifiers may themselves be targets for therapeutic intervention.

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**PRIORITIES**

- Characterize in a diverse population of HIV-infected children and adults the distribution of the replication-competent virus in all tissues.
- Characterize the impact of host immune environment on size, distribution and inducibility of the reservoir, focusing on most relevant tissues.
- Determine in animal models, and ultimately humans, the impact of immune-modifying drugs on latency reversal.
- Develop therapies that enhance the capacity of the immune system to target and eliminate virus-producing cells during ART.
- Develop therapies that durably enhance capacity of the immune system to control residual HIV in the absence of ART.
MODELS FOR HIV CURE OR SUSTAINABLE REMISSION AND PAEDIATRIC HIV CURE

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1. BACKGROUND

Potential clinical studies on HIV remission may involve approaches with limited or no human safety data and with unanticipated toxicities and, in some cases, with known toxicities. Combinations of different therapies will likely be needed in order to achieve HIV remission. Such combinations have potential for greater risks, but those must be balanced against the increased prospect of gain for the study participants and for other patients.

Important considerations surrounding long-term follow up of HIV remission include the risk of HIV-associated morbidity and mortality, which may persist despite virologic remission. This is exemplified in elite controllers who naturally control HIV to undetectable plasma viral load levels, yet experience excess hospital admissions (particularly for cardiovascular causes) compared with individuals on ART. Additionally, HIV remission might not completely eliminate risk of HIV transmission. It is expected that individuals who achieve HIV remission with undetectable viremia should have no greater risk of onward transmission than those who have clinically undetectable levels on ART.

Animal models involving experimental AIDS virus infection of non-human primates and humanized mice provide numerous important experimental advantages, including the ability to control experimental variability, definition of the identity, size, timing and route of the virus inoculum, flexibility of experimental and therapeutic interventions, and extensive tissue sampling, such as elective necropsy. These models are important contributors to the overall research effort aimed at achieving HIV remission. Studies utilizing animal models have informed the field about the rapidity of reservoir establishment, the anatomic and cellular components of the reservoirs, and the responses to novel interventions.

2. WHAT ARE THE GAPS IN OUR UNDERSTANDING?

Many factors limit our ability to define the conditions favouring viral control off ART. They include our incomplete understanding of the size, cellular and anatomic location, and dynamics of viral reservoirs. Of particular importance is our partial knowledge about the impact of the timing of ART initiation on reservoirs and the immunologic and virologic factors influencing reservoir formation. Interventions targeting viral reservoirs may vary as a function of the timing of ART initiation and of the resulting viro-immunologic milieu.

Individuals who initiate ART earlier may be expected to have smaller HIV reservoirs with less sequence diversity, potentially posing less of a challenge for eradication or control, especially with immunotherapeutic strategies. However, unless the daunting goal of viral eradication can be achieved in such individuals, immune control through native responses or immune-based therapies will likely be necessary for maintaining remission after ART interruption. Individuals initiating ART in chronic infection will likely require more extensive combinations of therapies to achieve HIV remission. These may include purging or repressing reservoirs coupled with immune-enhancing strategies and/or gene therapies. These hypotheses remain untested, however.

A consensus definition of “early ART” could accelerate HIV remission research by facilitating comparison of outcomes across cohorts and with various interventions in cohorts initiating ART at different times after infection. Such a definition could also help clarify the impact of ART initiation close to time of acquisition of infection on the potential for achieving off-ART remission in animal models and in infected persons.

Constructing a standard operational definition of “HIV remission”, for use in those research and community communications where “remission” is the preferred concept, could promote cross-talk between research studies and across disciplines, and improve community literacy. Clinical trials should address clinical, CD4, inflammation...
and residual virus outcomes to provide standardized assessment of any case of HIV remission achieved in the trials. A proposed operational definition for “HIV remission” (also known as “functional cure”), for use in both clinical research and community communications, includes the following criteria:

- Clinically undetectable plasma viral load at a specified level (<20 or <50 copies/ml) in the absence of either antiretroviral or immune-based therapy, for a minimum of six to 12 months after treatment interruption
- No clinically meaningful decline in CD4+ T cells during that period
- Absence of HIV-associated disease progression during that period
- Low-level residual virus (e.g., detectable cell-associated HIV DNA or HIV RNA or low-level plasma viremia <50 copies/ml detectable by ultrasensitive assays) and incomplete immune recovery may remain present
- In the specific context of reporting clinical trial results, the relevant threshold for detectable viremia and the duration off treatment should be specified as stated for the individual clinical trial protocol. One potentially useful reporting measure that can standardize reporting across clinical trials is “Viral Suppression Off-Treatment” or VS\(_x\)OT\(_y\), where \(x\) represents the threshold for detectable viremia and \(y\) represents the duration off treatment in months. An example is VS\(_{50}\)OT\(_{12}\), a designation specifying viral suppression <50 copies/ml while being off treatment for 12 months (Forum for Collaborative HIV Research, HIV Cure Project).

In these clinical trials, ongoing frequent viral load monitoring is critical in order to assess the typical duration of HIV remission under various strategies, and to allow resumption of ART promptly upon any recrudescence of HIV. The ongoing need for monitoring affects costs and study participants’ quality of life, and requires consideration as well. There are potential unknown consequences to being off ART as demonstrated by the START trial in which people with CD4+ below 500 cells/mm\(^3\) for an extended period of time had higher morbidities and mortalities, and immune activation.\(^{126}\) The possible risk of onward transmission after viral rebound in trials involving analytical treatment interruption (ATI) calls into question whether pre- or post-exposure prophylaxis should be offered to partners during this period. Biomarkers other than HIV RNA to predict ATI risks should also be explored.

3. RECOMMENDATIONS FOR FUTURE RESEARCH

Animal models can aid with: characterizing the mechanisms responsible for reservoir establishment and persistence; evaluation of safety and proof of concept in vivo activity of promising, but unproven and potentially risky, interventions (including combination interventions); assessment of candidate biomarker and intervention mechanisms of action studies, facilitated by extensive tissue studies; and evaluation of models to address different clinically relevant questions and the utility of each, including perinatal infection.

Important research questions exist around how best to design clinical trials to evaluate interventions for HIV remission. For interventions that are experimental and carry substantial risks to the participants with little or no individual benefits, careful consideration of the sample size, expected effect size and statistical power to demonstrate the potential effects is essential. For early-phase cure-related trials, animal models and small proof-of-concept human studies may be preferred, and their outcomes could inform the need for larger trials.

There is, however, a risk of rejecting therapies with modest benefit owing to an underpowered study. There are also important considerations concerning the ideal clinical groups for trials. A heterogeneous population that includes people of different age, sex, timing of infection and ART, and socioeconomic status could allow the exploration of benefits and risks across groups, but also compounds the challenge of detecting potential favourable effects
in a subset if the sample size is inadequate. Early-phase trials with a small sample size and a more homogenous population may result in a higher chance of detecting the benefits of the interventions, accepting that generalizability will be limited and subject to follow-up studies. Collaborating with experts in other fields, especially oncology, transplantation and hepatitis B, could provide new insights in mechanisms and interventions for HIV cure.

Clinical research studies should clearly define outcomes of interest and calibrate expectations accordingly. Because biomarkers for HIV remission are unknown, selecting the appropriate study endpoints is inherently challenging. Candidate predictive biomarkers for viral recrudescence will ultimately need to be validated by outcomes in treatment interruptions in adults and children. Testing of invasive or potentially high-risk interventions intended to bring about HIV remission should take into account the overall medical and non-medical risks and benefits for individual trial participants, and potential broader societal benefits. Many of the studied strategies, in particular gene-based therapies and vaccines that involve gene modifications or administration of live-viral vectors, could pose potential unforeseen long-term risks, including unknown risks to offspring. All should be reflected not only in researchers’ and review bodies’ decisions on whether to proceed with given protocols, but also in informed consent forms, process and comprehension testing.

Early, thorough ethical studies to identify research strategies that may lead to identifying contributors to HIV remission yet remain ethical toward study participants are critical. Lessons from early-phase cancer studies and first-in-human studies, as well as ingenious new solutions, may help ensure that early-phase HIV remission and cure studies remain ethical. It is essential to apply the findings of these ethical studies in HIV remission research design and overview. Scientific alternatives to including patients with otherwise good prognoses should be explored. Finally, it is critical to find optimal strategies to engage key stakeholders in research designs to identify types of (study) intervention that may lead to HIV remission acceptable to key stakeholders.

Communicating the concepts of HIV remission to the study participants and the larger community is also challenging. The goal of achieving HIV remission might turn out to be less exciting for people living with HIV than that of a cure. The many clinical, immunological and virological components of HIV remission might complicate effective communication of the complex notion of remission. Another area of research is the decision-making process by potential research participants in trials that test potentially risky interventions or include invasive procedures, such as tissue biopsies. Improved understanding of how benefits for individual trial participants and potential broader societal benefits play a role in decisions to join or decline these trials could inform engagement of future participants.
MODELS FOR HIV CURE OR SUSTAINABLE REMISSION:

PRIORITIES

- An ethical balance between scientific gain and study participants’ interests in testing invasive or potentially risky interventions intended to bring about HIV remission will heed overall medical and non-medical risks and benefits for individual participants, and the broader societal benefits.

- Iterative animal and clinical studies will be critical for understanding the anatomic and cellular components of the reservoirs, the responses to novel interventions and the utility of candidate biomarkers for predicting viral recrudescence.

- Understanding the impact of timing of ART on HIV persistence and remission would require in-depth characterization of the immunologic and virologic profiles at the time of ART initiation. An important question is whether interventions should be selected and studied based on timing of ART initiation.

- In many settings, the term “remission” should be prioritized over “cure”. Constructing an operational definition for HIV remission, for use in both clinical research and community communications, could facilitate cross-talk between research labs and across disciplines, and improve community literacy.

4. SPECIFIC CONSIDERATIONS FOR THE PAEDIATRIC POPULATION

Worldwide, almost 4 million children are living with HIV, and 250,000 are infected every year. Children face the prospect of life-long ART and the added challenges associated with antiretroviral drug treatment through childhood and adolescence (e.g., limited appropriate ART formulations, poor adherence). HIV remission represents an especially desirable goal for the paediatric population, and research towards this goal should be a priority. Perinatal HIV infection offers a unique opportunity to assess prompt control of HIV replication because of the known timing of HIV exposure through maternal infection. Immune tolerance in infancy, lower immune activation compared with adults, and the slow pace of T cell memory development have the potential to favourably impact HIV reservoir size following early interventions. Restricting HIV persistence may afford favourable profiles for eliminating latently infected cells.

Early therapy (defined as ART initiation before three months of age) is the recommended standard of care worldwide, regardless of clinical and immunological parameters. However, lack of point-of-care diagnostics makes early infant diagnosis challenging in resource-limited settings. Nevertheless, early treatment of perinatal infection leads to unique virologic and immunologic outcomes, including reduced frequency of latently infected cells. Early treatment is also strongly associated with the lack of HIV-specific immune responses, which opens a promising pathway for immune-based interventions: in the absence of autologous HIV-specific immune responses, responses to immunotherapeutic HIV vaccines or pharmacokinetics of HIV-specific monoclonal antibodies can be more clearly defined than in adult intervention trials.

The major knowledge gaps for perinatal HIV infection are in understanding the mechanisms of latency in infants and children. The dynamics of latency in children are probably different than those in adults, owing to a number of factors, such as the types and numbers of target cells, efficiency in clearing HIV-infected cells, and pharmacokinetics of...
ART in blood and tissues. Little is known about the development of the new-born and infant innate and adaptive immune system, and about the role of immune activation, homeostasis, inflammation and viral and host factors in the establishment and maintenance of HIV reservoirs. Early ART can preserve normal development of B and T cells, as demonstrated by the ability to mount immune responses against childhood vaccines.

However, there is a lack of understanding about the development of HIV-specific B and T cell immunity including neutralizing and non-neutralizing antibodies, and effector and polyfunctional T cell responses. Development of techniques for virologic and immunologic characterization that require small blood volumes is critical to advancing paediatric cure research. Similarly important are non-invasive methods to investigate tissue sanctuaries of HIV. The potential scientific gains from studies involving vulnerable populations, such as children, for whom decisions are made by proxies, should be balanced against the need to protect them from untested remission interventions and invasive investigations, such as tissue biopsies and lumbar puncture. Infant animal models could be used to fill gaps and limitations in paediatric HIV pathogenesis and mechanisms of interventions, as exemplified by the successes in prevention of mother-to-child transmission with antiretroviral drugs.

We can better characterize therapies in perinatal infection because of known timing of infection and the ability to initiate ART relative to known timing of infection. Moreover, the lifetime of ART and its potential toxicities make immunotherapies more compelling in this population relative to adults. However, in order to justify and design intervention studies in children, it will remain important to understand differences in the pathogenesis of HIV persistence in perinatal versus adult infections. Close collaborations between adult and paediatric cure researchers would enhance these understandings and advance HIV cure research for children and adults.

**RESEARCH IN NEONATES, INFANTS AND CHILDREN:**

**PRIORITIES**

- Understand the development of the innate and adaptive immune system in the systemic and mucosal compartments.
- Understand latent reservoir dynamics and factors associated with establishing and maintaining latency, including the CNS as a reservoir.
- Understand pre-existing immune responses against HIV and the role of immunotherapeutics in eliminating latently infected cells.
- Identify ways to appropriately conduct HIV cure research in children, including studies that involve new interventions, invasive procedures and ATI.
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1. BACKGROUND

There is growing interest in the potential of gene and cell therapies to treat HIV infection. This has been driven in part by recent technological advances and successes in other disease areas, especially cancer, together with the paradigm-shifting outcome for the Berlin Patient.60 This individual was cured of HIV as a result of receiving hematopoietic stem cell (HSC) transplantation as part of a leukaemia treatment from a donor whose cells were HIV resistant by virtue of being homozygous for the CCR5Δ32 deletion. Mimicking this approach, most gene therapies for HIV to date have been based on engineering a patient’s own (autologous) cells to confer HIV resistance.10 For example, mature CD4+ T cells, or the HSC precursor cells that give rise to them, can be genetically modified ex vivo in order to delete or block expression of the CCR5 gene.141 Alternatively, genes can be introduced into cells, using such vectors as lentiviral vectors, that encode proteins or RNA species that block HIV replication by targeting various stages of the viral lifecycle.142

In addition to these cell protection strategies, gene therapies are being considered as a way to directly remove HIV-infected cells and persistent reservoirs (Figure 3). For example, cells could be engineered to express toxins or suicide genes that are induced upon infection with HIV, effectively preventing spread of the virus by rapidly killing any infected cells.143 Alternatively, integrated HIV genomes could be targeted for inactivation using engineered nucleases, such as zinc finger nucleases (ZFNs) or CRISPR/Cas9.144 In addition, in the same way that engineered T cell receptors or chimeric antigen receptor T cells have proven successful against certain cancers, immune cells could be modified to recognize and destroy HIV-infected cells.145 Finally, cells could be turned into factories for the long-term production of anti-HIV molecules, such as broadly neutralizing antibodies146 or CD4/CCR5 mimetics.30,147 Gene therapies lend themselves to combinatorial approaches and could additionally be used in concert with strategies aimed at boosting immune responses, such as therapeutic vaccination.

Several recent clinical trials have been sponsored by biotech companies and performed in collaboration with academic and clinical partners. These include lentiviral vectors to express a CCR5 targeted shRNA and the peptide C46 entry inhibitor,148 or the use of ZFNs to disrupt the CCR5 gene open-reading frame.47 At the same time, assays to characterize patient baseline status and responses are becoming increasingly sophisticated, and trial participants are undergoing more informative types of sampling during therapies. It is therefore likely that ongoing and near-future trials will provide new information to further inform these approaches.

FIGURE 3.10 USING TARGETED NUCLEASES AGAINST HIV. Targeted nucleases, such as zinc finger nucleases and CRISPR/Cas9, provide more precise methods of gene therapy. They create site-specific DNA breaks, whose subsequent repair by the non-homologous end joining (NHEJ) pathway can be exploited to disrupt a gene, such as CCR5 or even an integrated HIV genome. Alternatively, repair can occur through homologous recombination, and a co-introduced DNA homology template can be designed to create small mutations in host genes or direct the site-specific insertion of an anti-HIV gene.
2. WHAT ARE THE GAPS IN OUR UNDERSTANDING?

There are several practical questions for HIV gene and cell therapies that are currently unresolved:

a. Which are the best cells to engineer to achieve a durable suppression of HIV replication?

Therapies that aim to create HIV-resistant CD4+ T cells can achieve this by engineering the T cells themselves or the precursor HSC that give rise to these cells in vivo. It is not known if the potential (but not yet proven) advantages offered by stem cells of longevity, and the ability to modify multiple cell types, including myeloid cells, will outweigh the greater technical challenges of working with these cells. Other factors to consider include the risk of insertion mutagenesis events if integrating vectors are used, which may be of greater concern when a long-lived stem cell is engineered. Also of concern are the cyto-ablative conditioning regimens that are currently needed for HSC therapies.

b. Is providing HIV-resistant cells necessary and/or sufficient for a cure?

The positive outcome for the Berlin Patient stands in contrast to the less successful long-term outcomes for the so-called Boston Patients. These two individuals also underwent HSC transplantation for blood cancers, although they remained on ART and did not receive cells from a homozygous CCR5Δ32 donor. Despite achieving undetectable levels of HIV post-transplantation and being able to prevent HIV rebound for an extended period of time after withdrawal of ART, they were ultimately unable to maintain suppression.

Although these observations suggest an essential role for the CCR5-negative donor cells received by the Berlin Patient, it is unknown if other aspects of his treatment were also essential for his HIV cure. This includes aspects of his therapy that are not as readily recapitulated by gene and cell therapy approaches. For example, the intensely cyto-reductive treatments he received to kill his leukaemia may have been needed to also deplete the persistent reservoir to a level that could be suppressed by the HIV-resistant donor-derived cells. Alternatively, the allogeneic (donor) cells he received may have contributed an essential anti-reservoir effect, in much the same way that allogeneic cells contribute anti-cancer effects, through graft-versus-host mechanisms. If this proves to be the case, it is not yet clear if it will be possible to boost the positive graft-versus-reservoir effects without incurring unacceptable graft-versus-host complications. Also unknown at present is whether these effects involve the same or different effector cells; the role of NK cells in particular is not understood.

c. The role of a selective advantage for engineered cells or their progeny.

With current technologies, the percentage of gene-modified cells created will represent a minority of the cells in the body. This is especially the case in non-cancer recipients, who do not receive highly depleting treatments before transplantation. However, it is also expected that there could be some selection for engineered HIV-resistant cells by the virus itself, although this may not be effective if ART is maintained and there is little/no replicating virus in the patient. Alternatively, such selection could be temporarily activated by a structured treatment interruption, although how long a period of uncontrolled virus replication would be required in order to select for a sufficient number of modified cells is unknown.
d. Can gene therapy produce an “immune-boosting” effect?

It is reasonable to consider that any therapy creating HIV-resistant immune cells should also lead to an increase in anti-HIV immune responses. However, this is still not known.

In a related point, it may be possible to improve immunological therapies using a simultaneous gene therapy aimed at enhancing the action of effector cells and innate responses. In some ways, this can be seen as a parallel approach to enhancing the immune response by treatment with drugs, such as those acting on immune-checkpoints.

e. Can gene/cell therapies be effective against HIV persistence in cells other than T cells, including tissue-resident myeloid cells?

This question includes consideration of whether any conditioning regimens that might be used to promote engraftment of engineered cells could also ablate different reservoirs, including long-lived macrophages and cells in the CNS.

3. WHAT CHALLENGES HAVE TO BE OVERCOME TO ADVANCE IN THIS AREA?

a. The challenges of appropriate pre-clinical studies and animal models.

The sophisticated nature of these treatments means that finding suitable animal models is difficult. Currently, gene and cell therapies are modelled in non-human primates, as well as in humanized mice (mice that are transplanted with human HSC). However, it is not yet known how predictive such models will be. There are significant challenges mimicking the human clinical situation (and especially time on ART) in an experimental model, and the additional steps of HIV infection and cell transplantation make the experiments technically challenging and very long term. Beyond the research goals described elsewhere in the document to standardize non-human primate (NHP) models of HIV suppression, latency and reservoir measurements, consideration should be given to what the appropriate approaches for NHP studies of gene and cell therapies are.

b. Concerns about the use of chemoablation to enhance HSC engraftment.

A major safety concern for autologous HSC therapies in non-cancer patients is attaining a balance between the toxicity of the chemoablation used versus the ultimate efficiency of engraftment of the cells, especially when such agents as busulfan and other alkylating agents are used. What level or type of “conditioning” is justified in otherwise healthy patients and in less healthy patients? Other less toxic or more specific approaches that are being considered are based, for example, on antibody-based cell depletion.

c. Is a period of viremia going to be required to select for modified CD4+ T cells?

As described, a treatment interruption with the goal of selecting for engineered cells may be desired. However, it is not known if this should be different from the design of an analytical treatment interruption undertaken to determine if reservoir reduction has occurred. Furthermore, will this period differ if using stem cell or CD4+ T cell infusions? How will the ART restart point be determined?
d. How will we identify the best patients for these therapies?

The newness of these approaches means that clinical trials will often start with patients with poor responses or low tolerance to ART. It is likely that parameters, such as baseline levels of inflammation, will emerge that could predict greater likelihood of success or failure of cell-based therapies.

e. Are we getting all the appropriate data from gene- and cell therapy-based clinical trials?

Trials will benefit from guidelines and consensus about the assays and measurements to be collected. It is not yet clear how to coordinate this, but we should make sure that we are setting up trials in a way that will gather data that is readily comparable to allow such evaluations. This also raises issues of bio-banking, sharing material/data and harmonizing data collection, including from industry partners. The sophistication of these approaches may mean that it is difficult to compare small trials undertaken at different places.

f. How will we know when we have succeeded?

Given the experiences of the Boston Patients and the Mississippi baby (HIV rebound after months or years of undetectable HIV), it is clear that even if transplantation of genetically modified HSC or T cells resulted in the apparent elimination of detectable HIV, such patients will require long-term observations off ART in order to determine if HIV has truly been eradicated. Monitoring will likely be similar to the current situation with cancer patients, with very close initial observation, moving to longer intervals, with an awareness that recurrence of the disease could still occur at a later date. This also raises the question of what level of HIV elimination will be required to claim success.

g. Public perception.

Finally, there are some general issues with the perception of these therapies by both patients and the public, and the practicability of implementing these therapies, especially in resource-poor countries. Put in the simplest terms, how do we make these types of treatments safe, scalable and cheap and, importantly, meet patient needs? Some of the same problems will also be factors for other approaches in HIV cure research. It will also be important to recognize that there may be a completely different view of risk/appeal of gene and cell therapies in different settings. Finally, we need to be sensitive to concerns about the use of resources, and the idea that these are currently only feasible in the developed world.

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4. RECOMMENDATIONS FOR FUTURE RESEARCH

a. Explore the potential of engineered T cells to eliminate HIV-infected cells.

The field of T cell engineering is moving rapidly, with great successes in the area of immuno-therapies for cancer. In a similar approach, it is possible that engineering T cells to express modified TCRs, or chimeric antigen receptors (CARs) recognizing HIV antigens, could provide control of HIV or reduce the reservoir. Other immune cells are also being developed as candidates for such engineering.
Targeted nucleases, such as ZFNs and CRISPR/Cas, have been shown to be able to disrupt integrated HIV genomes in cell culture models, but their application in patients will require methods to deliver them to infected cells in vivo. This includes the challenge of delivery to rare, latently infected cells, which do not express any signs of HIV infection. Achieving in vivo delivery is a challenge for the field of gene therapy in general.

c. Apply methods to boost immune responses in combination with gene/cell therapies.

It is uncertain if genetically modified HSC or T cells, although themselves resistant to HIV infection, could mediate eradication of latently infected cells. Thus, some additional mechanisms will likely be required to control or eliminate the reservoir, while the transplanted HIV-resistant cells protect against re-infection. Cell and gene therapies could therefore be combined with other treatments that boost HIV-specific immune responses, including novel therapeutic vaccine strategies, drugs modulating T cell responses, such as PD-1 inhibitors, and “kick” drugs to reactivate the persistent virus. There are already some indications that engineered HSC-derived CD4+ T cells or peripheral T cells can boost the endogenous immune system to control HIV, but this has to be understood better.

d. Investigate the consequences of chemoablation used to enhance HSC engraftment.

Although chemoablation increases the efficiency of engraftment, there are concerns about its toxicity, especially when agents, such as busulfan and other alkylating agents, are used. One mitigating approach is to do such studies in cancer patients first (e.g., AIDS lymphoma), but this patient population is small and getting smaller. Therefore, more investigation is needed into the long-term effects of such treatments since the risks of ablation in the autologous gene therapy setting are still unknown. Specifically, it is possible that cells could be damaged and undergo mutagenesis, without the safety net that an allogeneic transplant provides that such damaged cells would be killed.

e. Develop methods to enhance HSC engraftment without chemoablation.

These include safer (non-mutagenic) methods of conditioning and the possibility of positive selection for engineered cells in vivo, post-transplantation.

GENE AND CELL THERAPY:

PRIORITIES

- Explore the potential of engineered T cells to target HIV-infected cells.
- Develop methods to deliver gene therapies to reservoir cells.
- Develop methods to boost immune responses in combination with gene and cell therapies.
- Understand the consequences of chemoablation to enhance HSC engraftment.
- Develop alternatives to chemoablation to enhance HSC engraftment.
# Novel Biomarkers and Technologies to Analyse and Quantify HIV Reservoirs

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1. BACKGROUND

In the search for a cure of HIV infection, biomarkers are needed in order to understand the biology of HIV persistence on ART, to demonstrate the efficacy of interventions that may or may not directly reduce the HIV reservoir, and, most importantly, to predict the duration of ART-free remission. In addition, a long-term goal is to standardize assays of relevant biomarkers across multiple laboratories; it is important that the assays are reproducible and accurate, and are available in both high- and low-income countries.

There are several assays available that can be used to attempt to quantify HIV persistence under ART (Figure 4). The current gold standard for quantifying infectious virus that persists on ART is the quantitative viral outgrowth assay (QVOA), which requires a large number of resting CD4+ T cells, usually acquired from leukapheresis product or a large volume blood draw (160-180ml). Using this assay, the frequency of latently infected cells is in the range of one per million resting CD4+ T cells. The assay is labour intensive, expensive and not easily standardized across multiple laboratories. As a consequence, the reproducibility of the QVOA across laboratories has not been adequately assessed, although validation studies are in progress. Recent advances in measuring inducible virus have included a similar limiting dilution format, but measuring production of cell-associated multiple-spliced (MS) RNA (tat-rev induced limiting dilution assay, TILDA) or release of viral RNA in supernatant and amplification of persistent infectious virus using a humanized mouse model – the murine viral outgrowth assay (MVOA).

The frequency of latently infected cells carrying replication-competent virus measured by QVOA is significantly lower than the frequency of cells harbouring intact non-induced proviruses. Intact non-induced provirus occur at an approximate frequency of 60 per million CD4+ T cells and represent potentially infectious virus that is non-responsive to a single round of T cell activation in vitro, but may reactivate in vivo after treatment interruption. A subset of intact non-induced proviruses may never be activated because of specific integration site, condensed chromatin structure or other irreversible epigenetic mechanisms. Quantifying intact non-induced virus requires full-length proviral sequencing and cloning, and thus is not currently suitable for clinical studies.

HIV-infected cells can also be quantified using PCR-based assays. Total or integrated HIV DNA are both high throughput assays that are more easily standardized; however, they overestimate the number of latently infected cells because most proviruses that persist during ART have lethal mutations or deletions. Quantification of low-level plasma viremia by qPCR using the single copy assay (SCA) has been shown in some studies to correlate significantly with QVOA, but not in others. It remains unclear how frequently SCA detects infectious virus. New ways to identify the location of virus integration, although technically difficult, have demonstrated the important role of clonal expansion of infected cells in HIV persistence during ART.

It is possible that measuring the immune response to HIV could be a more sensitive strategy to detect residual viral replication than measuring the virus itself. The avidity and concentration of HIV antibodies appears to change with declining numbers of latently infected cells, and markers of immune activation (including T cell expression of HLA-DR and CD38 and PD-1) have been shown to correlate with the number of latently infected cells in multiple studies.

Biomarkers are also key to quantify the effects of interventions to perturb the reservoir. The most common use has been to quantify a response to a latency reversing agent (LRA). The potency of LRAs that induce HIV transcription has been quantified by measuring changes in cell-associated unspliced (US) HIV RNA. Other viral intermediates may also be important, including various spliced and US transcripts. However, recent sequence analyses of cell-associated HIV RNA revealed that >30% of this HIV RNA is hypermutant.
Production of detectable plasma virus as detected by SCA has been observed following some but not all LRAs in vivo. Simultaneous activation of HIV transcription and elimination of recently activated latently infected cells could potentially abrogate any measurable increase in plasma HIV RNA, and this may be important when assessing combination shock and kill strategies in the future.

No biomarker has been identified that can predict time to viral rebound following ART discontinuation or the duration of ART-free remission. Recent data suggest that cell-associated US HIV RNA, HIV DNA, immune function including expression of immune checkpoint markers or differences in NK cell function may predict the duration of ART-free remission. Additional prospective studies to verify these findings are needed.

Finally, it is important to emphasize that HIV-infected cells are found in multiple T cell subsets, activated and resting T cells, myeloid cells and in such tissues as the lymphoid tissue and gastrointestinal tract. Therefore, it is likely that measurement of virus in circulating total CD4+ T cells in blood may not adequately reflect the frequency or transcriptional activity of the total body reservoir of infectious virus on ART.

FIGURE 4 ASSAYS USED TO QUANTIFY HIV PERSISTENCE ON ART. The frequency of cells that produce infectious virus is only a subset of cells that are infected with intact (highlighted in a red line) and defective genomes (total pool of infected cells). US = unspliced; MS = multiply spliced; QVOA = quantitative viral outgrowth assay; MVOA = murine viral outgrowth assay

2. WHAT ARE THE GAPS IN OUR UNDERSTANDING?

Major gaps in our current knowledge include the biomarkers that predict the duration of ART-free remission, the most sensitive assays to detect HIV persistence on ART, the relationship between markers of HIV persistence in blood and tissue, quantification of the reservoir in specific patient populations, and a marker for a latently infected cell. The latter would greatly facilitate the study of latently infected cells in vivo and ex vivo.
In the absence of predictable biomarkers of virologic rebound, assessment of HIV remission requires interruption of ART. Development of inexpensive, point-of-care assays to quantify HIV RNA in plasma that could be performed at home could play an important role in early detection of viremia rebound. It currently remains unclear if more invasive procedures to identify tissue reservoirs are required prior to treatment cessation.

Although it has been well described that latency occurs primarily in resting memory CD4+ T cells, it is now clear that latency can occur in multiple T cell subsets, including central, transitional and effector memory T cells, T follicular helper cells, naive T cell and stem cell memory T cells. Recent evidence suggests that effector memory T cells are more likely to contain virus with multiple mutations, compared with central or transitional memory T cells. The factors determining the differences in viral persistence in different T cell subsets, including half-life and proliferative capacity, is a clear gap in our current understanding of latency; improved markers could inform this area.

The relative contribution of infected activated CD4+ T cells in virus persistence is also unclear. Whether myeloid cells are a true long-lived infected reservoir on ART is also an important issue that will require far more sensitive tools that can detect virus, specifically in tissue, including lymph node, the CNS and the gastrointestinal tract. Advances in immunohistochemistry, sensitive DNA and RNA probes, and novel techniques, such as laser capture microscopy, will all be helpful in answering these questions.

Biomarkers of HIV persistence likely differ in specific patient populations, including children, adolescents, women, transgender populations and the elderly. Differences in T cell subsets in children who have a high proportion of naïve T cells or the elderly who have a high proportion of proliferating and senescent cells may determine the distribution of where and how virus persists. Differences in innate immune function in women and the effects of oestrogen on HIV transcription and maintenance of HIV latency in vitro suggest that biomarker development should also address the issue of sex differences.

Currently, there is no phenotypic marker of a latently infected cell. Multiple surface markers have been associated with enrichment for HIV, including PD-1, other immune checkpoint markers, high expression of CD2 and a range of chemokine receptors. Identifying a phenotypic marker for a latently infected cell that would ultimately allow for single cell analyses remains a critically important priority for the field.

3. WHAT CHALLENGES HAVE TO BE OVERCOME TO ADVANCE IN THIS AREA?

To advance the field of biomarker identification, several challenges have to be overcome. First, the performance characteristics of putative biomarker assays must be much better characterized through unbiased assessment of sensitivity, specificity, precision and accuracy through testing of blinded panels of pedigreed samples that are prepared and distributed by a central organization. Funding for these processes and engagement of academic and industry scientists in this endeavour are surmountable but complex challenges.

Second, the relationship between biomarkers in blood and tissue has to be clearly defined. Biomarkers in blood may be too insensitive to adequately represent the extent of HIV persistence in tissue. Indeed, both the Boston transplant patients and the Mississippi Child provide evidence that blood sampling alone, perhaps because there is a limit to how much blood can be collected, will be inadequate as a measure of HIV persistence. Less cumbersome and scalable means of sampling tissue reservoirs have to be achieved as a potential means of increasing the sensitivity of biomarkers of HIV persistence. Advances in imaging technology and stereotactic guided tissue sampling hold promise, but reduction of risk and simplification of the sampling process to that equivalent to phlebotomy are formidable challenges.
Third, any putative biomarker, no matter how technologically advanced or appealing in rationale, has to be validated as a predictor of the duration of ART-free remission. A major repository of biologic samples of various types (blood, body fluids and tissues) and a robust clinical database of patients who suspend ART until viremia rebounds, are essential to validate biomarkers of the duration of ART-free remission. Such samples and clinical data are essential for identification of predictive biomarkers, but to date, enthusiasm and support for such a resource has not been forthcoming. Without it, advances toward a cure will be slowed because of the inability to readily differentiate successful interventions that impact the duration of being off ART from those that do not. Engagement of public and private organizations in the process of biomarker identification and validation is paramount for progress.

4. RECOMMENDATIONS FOR FUTURE RESEARCH

Future priorities for biomarker research should encompass several complementary efforts:

a. Pursue more sensitive methods for detection of replication-competent proviruses that readily differentiate intact from defective provirus in both blood and tissues.

The methods may involve: nucleic acid detection of signature proviral sequences that are present only in intact proviruses and not in defective ones; high throughput, full-length single proviral sequencing to identify intact proviruses; or simplified virus outgrowth assays that induce the complete reactivation of persistent but intact proviruses, potentially using additional stimuli other than activation of the T cell receptor. Recent innovations in high-throughput analyses of single cells should be applied with the goal of quantifying rare cells with inducible, intact proviruses.

b. Discover non-virologic biomarkers that detect with high sensitivity the persistence of HIV in an infected individual.

Potential biomarkers of this type include the levels of antibody to specific HIV proteins, the affinity of antibodies for such proteins, or the frequency of B-cells responsive to specific HIV-1 antigens. Similarly, assays to assess the frequency of CD4+ or CD8+ T cells that are responsive to specific HIV-1 antigens should be sought as markers of HIV-1 persistence.

c. Investigate host transcriptional or metabolic signatures of continued innate or adaptive immune response to HIV-1 nucleic acids or proteins as sensitive markers of HIV-1 persistence.

d. Develop and evaluate the potential for animal models,

Such as humanized mice, and the MVOA to detect infectious HIV-1 in large numbers of human cells that exceed the practical ability to assay in cell culture systems.

e. Analyse the performance characteristics of putative biomarkers,

Including sensitivity, specificity, reproducibility and accuracy, using standardized, blinded panels of well-characterized specimens that have a range of the relevant targets (virologic or non-virologic targets) for assay detection and quantification. Impartial, centralized distribution of test panels and data analysis are critical components of the research infrastructure needed to accurately determine biomarker assay performance.
f. Develop a post-mortem repository of tissues from HIV-infected individuals on ART to better define the relationship between tissue based and circulating reservoirs.

g. Perform studies of time to rebound of viremia after temporary cessation of ART in persons treated at varying times after infection to populate a rich repository of biologic specimens that can be used to validate the ability of select biomarkers to predict the duration of ART-free remission.

It is unlikely that a single biomarker will predict the duration of ART-free remission. A composite marker reflecting the size of the reservoir and the potency of the immune response may potentially be required. This vital but precious resource should only be used to assess assays of biomarkers that show the most promise in terms of rationale and performance characteristics. The highest priority should be given to validating biomarkers that show predictive power in NHP models.

h. Create an infrastructure to support capacity in low-income countries to participate in biomarker development and evaluation and ensure that all assays are optimized for a diverse global population and to also detect common circulating HIV clades, in addition to subtype B.

Pursuit of the above priorities will require multidisciplinary research, sizeable consortia to identify and validate biomarker of the duration of ART-free remission, and long-term engagement of funding agencies. Piecemeal approaches that are restrictive in scope are unlikely to be successful.
## SOCIAL SCIENCES AND HEALTH SYSTEMS RESEARCH

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1. BACKGROUND

Social sciences and health systems research are an essential component of the broader initiative towards an HIV cure. Social sciences are the study of human society and social relationships and include a wide range of disciplines (e.g., sociology, anthropology, psychology, history). Health systems research is “the production of new knowledge to improve how societies organize themselves to achieve health goals.” This includes public policy, health systems and health economics research.

Given the preliminary nature of current HIV cure research, we now have a unique window of opportunity during which social scientists and health systems researchers may work synergistically with biomedical scientists in order to make contributions to HIV cure research. Social science can inform the design and implementation of clinical trials, shape community engagement programmes and project economically viable cure strategies. Robust social science research now can help prepare for subsequent cure research and implementation. This is a major opportunity for multidisciplinary, cure-related research.

All health interventions, including those related to HIV, may have unintended implications. Anticipating some of the unintended implications of HIV cure research, both harmful and beneficial, will provide useful information for the ethical conduct of clinical trials and early implementation that emerges from trials. Potential unintended harmful implications include uncertainty that fuels mistrust of HIV cure research or the development of a costly HIV cure strategy that is not economically viable. This may be more prominent among key populations who have heard about sham HIV cures. Potential unintended beneficial implications include less need for HIV stand-alone services and integration of HIV and routine health infrastructures, streamlining service implementation.

Social science research on HIV cure has the potential to guide meaningful community engagement, ensure ethical conduct of research, mitigate the risks of behavioural disinhibition and therapeutic misconception, enhance patient-physician communication, engage global key populations, ensure economic viability, and reduce pervasive HIV and sexual stigma. Health systems research can facilitate policy-relevant research synergies, assist with health systems preparedness, spur public-private collaboration, and inform effective community engagement strategies. Social science and health services research are sufficiently nimble to both shape the context of HIV clinical cure research and to be shaped by developments and scientific advances within the cure field.

2. KNOWLEDGE GAPS

There are several major gaps in our understanding of HIV cure and HIV cure research that social science and health systems research may address. Key among these are knowledge gaps about the motivations, perceptions and expectations of key players – particularly patients/study participants and providers/clinical researchers – and about the community and structural contexts in which both cure research and, ultimately, cure programmes take place.

Understanding HIV-infected individual experiences, preferences and expectations is critical for sustaining engagement in HIV cure research over time. However, little is presently understood about how HIV-infected individuals perceive and experience HIV cure research. To our knowledge, there has only been one study examining the experiences and expectations of HIV-infected individuals enrolled in HIV cure research, one quantitative survey of HIV-infected individuals, and one qualitative analysis of HIV-infected individuals. Further ongoing studies are addressing these issues.
Furthermore, HIV community advocates and civil society organizations have played a vital role in expanding HIV treatment access and are likely to play an important role in future HIV cure research and implementation. Nevertheless, this too has so far received little research attention. Additionally, the local context of HIV cure research may be different in some areas. For example, previous examples of ineffective HIV cures in South Africa, Zambia, Zimbabwe, the Gambia and Nigeria may influence how HIV-infected individuals perceive HIV cure research moving forward. The cost of delivering a cure and the opportunity cost would likely be different in these resource-constrained contexts. Yet, to date, none of the existing literature has focused on low-income country contexts that have a higher burden of HIV and would potentially have the greatest to benefit from a cure.

The perspective of physicians and other health providers is also important for HIV cure research. Physicians manage expectations about HIV cure research and are a trusted and critical source of information about HIV cure trials and ongoing scientific advances. Research about health professional perceptions, attitudes and beliefs on HIV cure research can help shape communication strategies, informed consent tools and related tools to mitigate unintended consequences, such as therapeutic misconception and behavioural disinhibition.

Beyond the individual perspectives of HIV-infected individuals and health providers, the HIV-infected individual/provider relationship is another important area of investigation. Qualitative research has shown that these relationships are critical in the context of HIV cure research. Relationships between HIV-infected individuals and their providers may influence communication, expectations and other key aspects of clinical trial recruitment and retention.

Finally, the dynamics of cure research are affected by the local policy/regulatory/legal context in which they occur, a context that may encourage or challenge the advancement of HIV cure trials. This context includes health financing, governance and policy as it relates to structuring, planning, managing and delivering services in resource-rich and resource-constrained settings. Further social science and health systems research is needed in each of these fields. There is little known about the trade-offs of the upfront costs and quality of life gains compared with the lifetime costs of ART. As such, the risk-benefit calculus associated with the development of an HIV cure is unclear. Some HIV cure strategies may have an early high risk of morbidity, or even mortality, but downstream, have high efficacy.

Addressing these knowledge gaps can help improve a number of clinically important outcomes, including obtaining ethics board and human subjects approval at the local level, recruitment of participants, retention and adherence of participants, HIV-infected individual acceptance of clinical trials, support and advocacy related to research, regulatory approval, and scale up and eventual implementation. Furthermore, little is known about the potential impact of social and legal contexts (e.g., HIV serostatus stigma, criminalization of HIV exposure and consensual same-sex behaviour) on HIV cure research.

3. CHALLENGES THAT HAVE TO BE OVERCOME

Social science and health systems research in support of HIV cure research face several challenges.

First, although strong social science research should link together the methodological perspective of anthropologists, sociologists and psychologists with the perspective of biomedical and clinical scientists who are organizing and leading clinical trials, the boundaries of traditional academic disciplines often constrain interdisciplinarity. These disciplinary boundaries are often reinforced by professional associations, disciplinary journals and discipline-specific methodological advances. This challenge is compounded because many social scientists and health systems researchers are focused on other HIV research priorities, such as treatment, vaccines and microbicides. Attracting social scientists to HIV cure
Second, most HIV cure trials are now being conducted in high-income countries and disproportionately enrol white men. This creates a relatively small group of stakeholders who are knowledgeable about the basic science, clinical progress, economic and policy implications and public health issues related to HIV cure projects. Engaging a broader scope of stakeholders in low-income country and middle-income country contexts and in contexts with high HIV prevalence will be important as clinical trials continue.

Several promising areas for future research are outlined here. This is not meant to be an exhaustive list, but these are several main areas that require further investigation.

a. Identifying and responding to HIV-infected individuals’ perceptions.

The voices of HIV-infected individuals have always been central to the HIV response, and this must extend to cure research. For many individuals with HIV infection, their serostatus has been the basis for making a number of decisions that influence health (e.g., serosorting and sexual behaviours) and wellbeing (e.g., participation in community groups and advocacy). How they understand the meaning of HIV cure is important because these beliefs may influence participation in clinical trials, trust in HIV service delivery systems, engagement in care and treatment, serostatus disclosure, and ongoing risk and protective behaviours. Serological status itself may not change after the development of a cure.

The personal, behavioural and social implications for HIV-infected individuals, their partners and their communities of participating in HIV cure clinical research warrant greater attention in the context of clinical research. Examples of this type of research include: research about how to effectively communicate the risks of HIV cure trials as part of informed consent (especially among individuals with acute HIV infection); qualitative research among HIV-infected trial participants and their partners about how participation in cure studies affect the participant’s HIV identity, sexual behaviours and social relationships; and research on how HIV-infected individuals understand (or misunderstand) ongoing HIV cure research and the meaning of an HIV cure in their own lives.

In addition to identifying and responding to the preferences of HIV-infected individuals in cure trials, there is also a need for further research among HIV-infected individuals globally who are not in trials. Although there are a growing number of HIV cure trials, most trials are in high-income countries. Will HIV-infected individuals participate in clinical trials if they believe that only the rich will have access to the cure? The preferences, attitudes and beliefs of non-participants are also an important factor in designing studies.

b. Measuring and increasing stakeholder engagement.

There are a number of stakeholders in cure research, including HIV-infected individuals, key affected populations, health professionals, scientists, funding agencies, international agencies, public health and regulatory authorities, pharmaceutical industries and civil society organizations. Together, this group will set the pace of developing and implementing an HIV cure. Stakeholders can play an important role in promoting new cures during early-phase clinical trials, late-phase clinical trials and implementation, and as part of retention programmes. The history of HIV intervention research shows how early stakeholder engagement along multiple levels can help increase the likelihood of success and mitigate failure.

However, there are many key research areas that require further investigation, including: better and more standardized tools for measuring stakeholder engagement and its downstream effects (e.g., changes in retention rates, recruitment pace, and changes in the composition of community advisory boards); optimal timing and
c. Clinical trial equity and inclusiveness.

In order to ensure an equitable distribution of cure strategies, it is essential to incorporate a diverse group of participants in terms of sex, ethnicity/nationality, location, age and other characteristics. However, optimal mechanisms and tools for achieving this inclusiveness are unclear. This field could benefit from understanding the experience of the HIV vaccine research field over the past several decades. Research opportunities in this realm include: community-based research on how to ensure inclusiveness and representativeness in HIV cure studies; and approaches to improve the likelihood of equitable implementation of and access to efficacious HIV cure strategies, especially in resource-limited settings.

d. Health systems research.

Modelling research could help researchers understand which individual cure strategy or group of strategies would be optimal to effectively create population-level effects. Information analysis can help inform which cure candidates would have the highest chance of having the largest impact at the population level for the longest duration. Pre-trial modelling could help identify influential short- and long-term cost-effectiveness outcomes and ensure that the trial is adequately powered to evaluate those outcomes. Initial cost-effectiveness studies have compared potential HIV cure approaches and identified combinations of efficacy and relapse rate leading to cost-effective and cost-saving strategies compared with ART. Both cost-effectiveness (i.e., will the strategy be worth paying for?) and budgetary impact research (i.e., will the strategy be affordable?) will be important, especially in resource-limited settings.

Value of information analysis determines the amount a decision maker would be willing to pay for information (e.g., the results of an HIV cure trial) prior to making a decision. This type of decision-analysis tool may help funders and other groups make decisions about which trials should be prioritized. Health policy research is needed to ensure that there is synergy between HIV cure and other existing HIV programme priorities. Health equity analysis to guide and monitor fair and ethical policy and decision making is important. Identifying who will pay for a cure and how prices will be established are also critical. Studies on how best to enhance public-private collaboration towards an HIV cure could alleviate some of the regulatory and logistical challenges associated with drug development.
<table>
<thead>
<tr>
<th>Trial Phase</th>
<th>Ethics</th>
<th>Participant Preferences</th>
<th>Stakeholder Engagement</th>
<th>Health Communications</th>
<th>Fairness, Equity, Disparities</th>
<th>Health Systems Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Apply rapid cycle improvement methods to develop understandable and workable IC processes</td>
<td>Careful attention to individuals’ goals and needs in deciding to participate in</td>
<td>Intensive education of key stakeholders in a few key settings from which patients will be recruited will be needed for trials</td>
<td>Exploratory research on the kinds of communications methods required to adequately inform trial participants and their partners and families about cure research and trial issues (e.g., optimal format, timing, delivery, context)</td>
<td>Decide on a relatively small handful of diverse but somewhat homogeneous groups to conduct phase 1 trials, contingent on HIV epidemiology</td>
<td>Modelling to help identify key CEA outcomes and ensure studies are adequately powered to assess them; pre-trial &quot;value of information&quot; analyses</td>
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<tr>
<td>2</td>
<td>Conduct study exit interviews to ensure that CQI methods are applied to the IC process so that they improve with each phase of the research</td>
<td>Same as above, as well as exit interviews or other formal methods to ensure that we understand enough about participants’ experiences to run effective phase 3 studies</td>
<td>Use lessons from above to widen and broaden the education and engagement of key stakeholders in preparation for phase 3 studies. Continue to test and improve stakeholder engagement methods</td>
<td>Continue exploratory research. Prepare for the more diverse populations in phase 3 trials and how their communications needs may be different</td>
<td>Expand very intentionally to a wider set of groups with an eye towards implementation in a very diverse group of participants in phase 3 studies</td>
<td>Modelling to help identify key CEA outcomes and ensure studies are adequately powered to assess them</td>
</tr>
<tr>
<td>3</td>
<td>Ensure that IC processes evolve to meet the needs of the broader group of participants who will be involved with phase 3 trials. Perhaps start to study the quality of the IC process</td>
<td>Develop and implement short, standardized methods to assess participants’ experiences in trial, both to be able to do CQI and to compare quality across sites</td>
<td>Apply methods developed in earlier phases to implement the kind of broad stakeholder engagement practices across many sites that will be required for multisite phase 3 studies. Study stakeholders to examine and compare engagement across sites</td>
<td>Implement the communications strategies developed during phases 1 and 2. Compare quality across sites. Test quality improvement interventions</td>
<td>Phase 3 studies would ideally be done in sufficiently diverse settings to allow reasonably well-powered, a priori subset analyses (e.g., men vs. women). Studies of rates of enrolments in different sites that serve different populations could be informative.</td>
<td>Research on workforce issues, access, CEA, budgetary impact</td>
</tr>
<tr>
<td>4</td>
<td>Research on shared decision making, public understanding of risks and benefits of therapy</td>
<td>Research could compare the quality of the treatment experience in a rollout or implementation phase with that during the previous phase</td>
<td>Develop and test public relations and social media methods to disseminate knowledge about cure issues</td>
<td>Experiment with public health and social media approaches to communications to reach the widest possible audience</td>
<td>Formal disparities research is appropriate in the phase 4 setting</td>
<td>Formal HSR studies are appropriate, such as how different structural features of health systems relate to better outcomes, CEA, modelling; impact of CEA on efficacy vs. effectiveness</td>
</tr>
</tbody>
</table>

IC = informed consent; CQI = continuous quality improvement; CEA = cost-effectiveness analysis; HSR = health systems research
SOCIAL SCIENCES AND HEALTH SYSTEMS RESEARCH:

PRIORITIES

• Carry out patient-focused research to understand the perceptions, attitudes and beliefs of HIV-infected individuals, their partners and communities towards HIV cure. This includes behavioural and social factors, such as medication adherence, HIV risk behaviours, disclosure and stigma.

• Measure stakeholder engagement in HIV cure research among a diverse group of individuals from a range of local contexts.

• Develop effective strategies for increasing stakeholder engagement in HIV cure research, particularly in low- and middle-income country settings.

• Determine optimal health systems and policy strategies to promote HIV cure research.

• Use decision analysis and related modelling strategies to optimize clinical trials, enhance HIV cure strategies and demonstrate budgetary impact.
METHODOLOGY

This second edition of the Global Scientific Strategy: Towards an HIV Cure was developed under the auspices of the International AIDS Society (IAS) to revise and update the original strategy released in 2012. The strategy was discussed and developed by the International Scientific Working Group, a global team of leading stakeholders, including basic scientists, clinical physicians, social scientists, ethicists and community leaders from around the world. The International Scientific Working Group was composed of a steering committee and six multidisciplinary subgroups, including input from medical ethicists in each subgroup.

The Global Scientific Strategy was discussed, developed and finalized at a series of in-person workshops and electronic discussions from September 2014 to March 2016. In addition to the discussions within the International Scientific Working Group, the Global Scientific Strategy underwent broad dissemination for a peer-review process incorporating comments and edits from a broad range of stakeholders, in keeping with the values of the International AIDS Society. These research recommendations represent the culmination of hundreds of hours of online and in-person meetings with community leaders, pharmaceutical company representatives, funders and regulatory agency representatives, as well as HIV researchers from low-income, middle-income and high-income country contexts. A number of non-HIV researchers were consulted on specific scientific issues.

The IAS is committed to promoting and encouraging research collaborations towards an HIV cure. At the same time, this responsibility is shared by a wide range of funding agencies, philanthropic organizations, businesses, scientific and medical communities, people living with HIV, and others around the world. The IAS and the authors of this Global Scientific Strategy hope that these recommendations will be useful for coordinating and accelerating research and priority setting to successfully develop an HIV cure.

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