

VIEWPOINT

HIV reservoirs: what, where and how to target them

Melissa J. Churchill, Steven G. Deeks, David M. Margolis, Robert F. Siliciano, and Ronald Swanstrom

Abstract | One of the main challenges in the fight against HIV infection is to develop strategies that are able to eliminate the persistent viral reservoir that harbours integrated, replication-competent provirus within host cellular DNA. This reservoir is resistant to antiretroviral therapy (ART) and to clearance by the immune system of the host; viruses originating from this reservoir lead to rebound viraemia once treatment is stopped, giving rise to new rounds of infection. Several studies have focused on elucidating the cells and tissues that harbour persistent virus, the true size of the reservoir and how best to target it, but these topics are the subject of ongoing debate. In this Viewpoint article, several experts in the field discuss the constitution of the viral reservoir, how best to measure it and the best ways to target this source of persistent infection.

Q *Historically, the HIV reservoir has been considered to consist primarily of infected long-lived memory CD4⁺ T cells, but several other T cell subsets, such as naive CD4⁺ T cells and T follicular helper (T_{FH}) cells, and even other cell types, including macrophages, have been proposed to contribute to this reservoir. In your opinion, what cell types constitute the viral reservoir, and why has it been controversial to expand this reservoir beyond memory CD4⁺ T cells?*

Robert F. Siliciano. Much of the confusion surrounds the use of the term ‘reservoir’. We have proposed that the term be defined in a practical way. From a clinical perspective, the only HIV reservoirs that matter are those that allow persistence of replication-competent virus in patients who are undergoing long-term, optimal antiretroviral therapy (ART). This is because HIV cure interventions will only be attempted in patients who have long-term suppression of viral replication under these conditions.

The only cell type for which the above criteria have been demonstrated is resting CD4⁺ T cells. Among resting CD4⁺ T cells, most of the latent virus is in cells of a memory phenotype. Various subpopulations

of memory cells, such as central and effector memory cells, harbour different frequencies of latently infected cells in different patients. Other cell types, such as macrophages, may be infected *in vivo*, but whether they can persist in an infected state for years in the setting of ART is not yet clear, so from a clinical standpoint, it is not clear whether these cells contribute to the reservoir.

David M. Margolis. The question uses the word ‘reservoir’, but it is really the concept of HIV latency, the greatest obstacle to eradication, that must be clearly defined. A truly latent virus — which, by definition, does not give rise to new viral particles — cannot be targeted by any immune effector mechanisms (natural or engineered) or therapeutic modalities (except for gene therapy enzymes that are not yet deliverable).

Latent infection must be rigorously defined as a cell that can be shown to lack the expression of viral particles at one moment in time, and later found to express infectious viral particles following exposure to an agent that reverses latency. It is clear that resting, central memory CD4⁺ T cells are the most numerous

latently infected cells and contribute the most to the reservoir of cells and tissues in which infection persists despite clinically effective ART. However, we and others have demonstrated that other types of lymphoid cells (such as naive CD4⁺ T cells, stem memory T cells and transitional memory CD4⁺ T cells) can be defined as latently infected in the rigorous way proposed above. We have also recently found that $\gamma\delta$ T cells can be latently infected¹. However, for all of these alternative lymphoid populations, it is still necessary to clarify whether latent infection is as durable in the face of stable therapy as it is in the central memory compartment. Preliminary studies suggest that this may not be the case, and therefore latent infection may not be as stable in these other lymphoid populations as it is in central memory T cells. Similarly, latent infection in non-lymphoid cells has not yet been rigorously demonstrated (as defined above) in cells from patients, or in animal models, that have achieved full suppression of viraemia over the course of several months. Therefore, it is currently unclear whether cells other than resting, central memory CD4⁺ T cells contribute to durable, persistent latent HIV infection.

Steven G. Deeks. There is no question that the memory CD4⁺ T cell population harbours nearly all (and perhaps all) of the replication-competent virus during ART. It remains unclear, however, whether certain memory cell populations are enriched for the virus. For example, there is an emerging story suggesting that T_{FH} cells, which reside in the B cell follicles of lymphoid tissues, are highly enriched for replication-competent virus. These follicles can thus serve as a relative sanctuary for the virus, as host effector cells and perhaps even antiretroviral drugs are unable to access these areas. Our group has also shown that memory T cells expressing immune checkpoint receptors (particularly programmed cell death protein 1 (PD1; also known as PDCD1), but there are others), markers of T cell proliferation (such as human leukocyte antigen DR (HLA-DR; a major histocompatibility complex (MHC) class II molecule)) and markers of T cell activation (such as CD38 and

C–C chemokine receptor type 5 (CCR5)) are enriched for HIV DNA and RNA.

The degree to which replication-competent virus persists in macrophages, naive T cells and other cell types during long-term ART remains controversial. Macrophages clearly harbour HIV DNA but whether this reflects active infection or phagocytosis of infected CD4⁺ T cells is actively being debated. As latently infected cells do not make HIV proteins, there is intense interest in identifying a host marker that is expressed (or perhaps downregulated) in such cells. This may or may not exist, but the identification of such a marker would have a huge impact on the cure field.

Melissa J. Churchill. Although it is clear that the primary HIV reservoir resides in memory CD4⁺ T cells, with numerous studies confirming that these cells contain latent replication-competent virus, other cell types and tissues are also known to be infected with HIV and could potentially

establish HIV reservoirs. For example, macrophages, microglia and astrocytes in the central nervous system (CNS) can become infected with HIV, and integrated HIV genomes have been detected in purified nuclei from such cells isolated from the brain tissue of infected patients². Furthermore, these are long-lived cells that have the potential to support latent infection. Assuming that these cells can persist in virally suppressed infected individuals who are undergoing ART, infected macrophages, microglia and astrocytes could contribute to the HIV reservoir. However, whether these cells can be reactivated to produce virus capable of reseeding the infection or instead cause a more localized neurological disease is unclear.

Importantly, it is also necessary to consider the more general question of what constitutes a viral reservoir. It is accepted that, in order to have a biologically significant role, infected tissues and cells must preserve a replication-competent form

of the virus that can potentially replenish the population of infected cells. However, this description may exclude sanctuary sites, such as the CNS, from being considered potentially important reservoirs. Specifically, tissue damage can occur in the CNS even without the production of replication-competent virus, predominantly attributable to the production of viral proteins, such as Tat³.

Ronald Swanstrom. First, it is important to distinguish between a latent reservoir and an active reservoir. A latent reservoir requires a long-lived cell to harbour HIV in an unexpressed (or under-expressed) state for years, as patients undergo ART. Therefore, any infected T cell type that can maintain itself either in a quiescent state or through periodic cell division (without reactivating the latent virus) can represent a reservoir, with resting CD4⁺ memory T cells providing the clearest example. By extension, any long-lived cells that express the CD4 receptor and the CCR5 coreceptor on their surface at sufficient levels to allow them to be efficiently infected could contribute to the reservoir. There are now examples where such cells clearly proliferate, which extends their lifespan; in some cases, this is due to insertional activation by the viral DNA. These cells represent clonal outgrowths with the same provirus that can be identified either by the insertion site in host DNA or by a distinctive DNA structure (such as a deletion). Furthermore, any cell type that can accumulate defective viral DNA is likely to include a small percentage of cells that have intact but quiescent viral DNA.

By contrast, an active reservoir, whereby virus numbers are maintained by replication in short-lived cells even in the face of suppressive ART, is another potential source of HIV. The weight of the evidence — including the observation that therapy intensification does not reduce low-level viraemia and that viral sequences do not evolve over time — argues against an active reservoir. However, these are negative results based on virus detected in blood; I think we have to acknowledge that we know much less about the potential for low-level viral replication in tissues. The most likely place for this scenario to play out is in the parenchyma of the brain, where macrophages and microglia could support replication of macrophage-tropic viral variants in an environment where drug concentrations are low. We have recently identified a person who seems to have

The contributors*

Melissa J. Churchill is a Burnet Principal Fellow at the Burnet Institute, Melbourne, Victoria, Australia, and Associate Professor of Medicine and Microbiology at Monash University, Melbourne, Victoria, Australia. She completed her Ph.D. at the University of Melbourne, Victoria, Australia, and postdoctoral training at GlaxoSmithKline (GSK) in Philadelphia, Pennsylvania, USA. She currently heads the HIV Neuropathogenesis Laboratory at the Burnet Institute where the focus of her laboratory is on HIV-1 infection of the central nervous system and tissue reservoirs.

Steven G. Deeks is Professor of Medicine in residence at the University of California, San Francisco (UCSF), USA. He has been engaged in HIV research and clinical care since 1993. He directs The SCOPE Cohort, a large clinical infrastructure aimed at enabling the study of HIV-associated immune dysfunction and HIV persistence during antiretroviral therapy (ART). In addition to his clinical and translational investigation, he maintains a primary care clinic for adults infected with HIV.

David M. Margolis is Professor of Medicine, Microbiology and Immunology, and Epidemiology, and the Director of the University of North Carolina (UNC) HIV Cure Center, Chapel Hill, USA. As a graduate of Tufts University School of Medicine, Boston, Massachusetts, USA, he trained in medicine at the New England Medical Center, Boston, Massachusetts, USA, in infectious diseases at the US National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland, USA, and did postdoctoral research on the regulation of HIV-1 gene expression at the University of Massachusetts Medical School, Worcester, USA. He continues to treat patients and carry out clinical studies at UNC while his laboratory studies interactions between HIV-1 and the host cell at the molecular level, seeking insights to enable the development of approaches to eradicate HIV-1 infection. David Margolis' homepage: <http://www.med.unc.edu/microimm/margolislab>

Robert F. Siliciano received his M.D. and Ph.D. from the Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. He did postdoctoral work at Harvard Medical School, Cambridge, Massachusetts, USA, before joining the faculty at Johns Hopkins University. He is a member of the Howard Hughes Medical Institute and Professor of Medicine and Molecular Biology and Genetics at Johns Hopkins University. His laboratory is interested in the characterization, quantification and therapeutic reduction of the latent reservoir of HIV-1.

Ronald Swanstrom received his Ph.D. from the University of California, Irvine (UCI), USA, and did his postdoctoral studies at the University of California, San Francisco (UCSF), USA. He then joined the faculty at the University of North Carolina (UNC) at Chapel Hill, USA, where he is now the Charles Postelle Distinguished Professor of Biochemistry and Director of the UNC Center For AIDS Research. His laboratory is interested in HIV-1 evolution, pathogenesis and reservoirs. Ronald Swanstrom's homepage: <http://swanstrom.web.unc.edu/>

*Listed in alphabetical order.

such an active reservoir, suggesting that these cells have the potential to support an active reservoir.

Q *Similarly to the debate over what constitutes the cellular HIV reservoir, there seems to be an ongoing discussion about what tissues harbour latently infected cells. For example, the gut-associated lymphoid tissue (GALT) seems to harbour latently infected cells, but whether cells in other organs, such as macrophages in the brain, contribute to the reservoir, is a matter of debate. In your opinion, what tissues constitute the viral reservoir and what is likely to be their relative contribution to disease reactivation once ART is stopped?*

S.G.D. The reservoir for HIV is primarily the memory CD4⁺ T cell compartment. These cells generally reside in secondary lymph nodes, the spleen and the gut mucosa. As one might expect, the vast majority of the virus resides in these tissues. The degree to which the virus is uniformly distributed across all lymphoid tissues remains unknown. The person-to-person variability in tissue distribution is also unknown. How age, gender, ART duration, viral subtype and other key factors influence the distribution of the virus has yet to be addressed in any prospective manner.

Some studies have suggested that the genitourinary system may be particularly enriched for the virus. The brain harbours HIV in untreated HIV disease, but the degree to which the virus persists indefinitely during treatment in this tissue remains undefined. Macrophages may be an important reservoir in these difficult to study tissues.

R.F.S. The latent reservoir for HIV in resting memory CD4⁺ T cells is widely distributed throughout the body, essentially wherever memory T cells are present. Although CD4⁺ T cells in the gut have high amounts of HIV DNA, it is less clear whether they harbour replication-competent virus. The distinction is important because the vast majority of HIV DNA detected by PCR is highly defective and should not be considered as part of the reservoir (see below). Isolating replication-competent virus from cells in GALT is complicated by the need to obtain tissue biopsies from patients on ART, and to dissociate the tissue and isolate viable cells in a sterile manner. These technical challenges have prevented adequate analysis of HIV persistence in the GALT. With regard to infected macrophages in the brain,

as discussed above, it is still unclear whether they can persist in an infected state for years in the setting of ART.

D.M.M. Emblematic of the ongoing debate, it is not clear that the demonstration of true virological latency has been established in GALT. Abundant HIV DNA can be found in the GALT of durably suppressed patients, and intermittent, anatomically scattered cells, or a nest of cells, expressing HIV RNA can be found. However, these are not latent infections — they are active infections. Some investigators term these cells the ‘active reservoir’, a term which I find confusing; a true reservoir cell must persist and the lifespan of the cells expressing HIV RNA (and presumably in some cases HIV antigen or particles) is unknown. It seems plausible that such cells may have been formerly latently infected, and upon transit into the GALT may have encountered an antigen or stimulus that induced the expression of virus, but this still lacks experimental demonstration.

As discussed above, we cannot rule out latent infection in cells of myeloid or other lineage, but direct and rigorous demonstration of true virological latency in such cells has not yet been achieved. In my view, the first priority is to make progress towards effectively and safely depleting the clearly identified latent reservoir within memory T cells. In doing so, the importance of other potential reservoirs may be clarified, as may the need for specific approaches to attack them.

R.S. This is a very difficult question to address in a convincing way. For example, investigators do not get to observe the initial event of virus being released from the latent reservoir (except perhaps with a biopsy). What can be observed is the rebound virus, which is amplified by replication after release from the latently infected cells, but the origin of this virus is hard to assess. Therefore, any tissue that has resting CD4⁺ T cells that harbour replication-competent virus could be the source of rebound virus. Furthermore, with the background of the robust reactivation of virus that we assume is coming from these latently infected T cells, other reservoirs, if they exist, will be hard to identify. Viral genetics could help with this problem. For example, we have recently observed that rebound virus in the blood is the typical R5 T cell-tropic HIV (which infects T cells expressing the CCR5 coreceptor and high levels of CD4) that dominates at all stages of infection.

This shows that the rebound virus was not primarily replicating in myeloid cells before entering the reservoir, as this would have selected for a ‘macrophage-tropic’ rebound virus, which would have the ability to infect cells with a low density of CD4. However, even R5 T cell-tropic viruses can infect macrophages with about one-thirtieth of the efficiency of the evolved macrophage-tropic viruses, so it is hard to say that there are no infected macrophages just because the virus is R5 T cell-tropic. To more closely study whether alternative sources, such as infected macrophages in the brain, contribute to rebound viraemia, we are studying viral populations in the cerebrospinal fluid (CSF), which are likely to originate from the brain. However, infected T cells trafficking from the blood are likely to present a high background when trying to detect such a reservoir, if it exists.

M.J.C. Determining the location and possible contribution of tissue reservoirs is an ongoing challenge for the cure field. For example, the CNS presents some unique challenges to cure strategies. There is no direct evidence that cells within the brain of ART-suppressed patients contain replication-competent HIV genomes that are potentially capable of producing virus. Conversely, there is no definitive evidence indicating that latently infected cells do not persist in the brain. It is well established that patients have productive HIV infection of macrophages and microglia, and restricted infection of astrocytes. The degree of infection of these cells has been demonstrated to correlate with CNS clinical disease, with brain-derived virus often detectable in the CSF. Furthermore, there is a growing body of evidence that HIV DNA is detectable in autopsy brain tissues isolated from patients who have died while receiving suppressive ART or in patients without any detectable viral load. However, determining whether these HIV genomes are replication-competent and capable of producing virus upon activation is problematic.

From clinical studies describing ‘CSF escape’, in which patients maintaining undetectable HIV levels in the plasma develop CNS disease, it is clear that these patients have an increased viral load in the CSF, which is probably seeded from the brain^{4–6}. In some cases, this virus is resistant to ART regimes controlling plasma viraemia. Therefore, these reports suggest that the CNS is potentially a persistent HIV reservoir. Whether this reservoir can lead to reseeded of infection in the periphery is unclear.

Q A parallel question has been what is the best way to measure viral reservoirs, as there are multiple assays available that measure different aspects of viral integration and reactivation. What do you think are the most informative assays that are available to measure the HIV reservoir, and why? Is there a 'gold standard' that should be used across different studies?

R.S. Clinical virology has depended on the ability to grow virus for 100 years, and in that tradition the viral outgrowth assay (VOA; in which resting CD4⁺ T cells are subjected to a single round of activation with a mitogen and the resulting amount of produced virus is measured) is the 'gold standard' for the presence of virus. However, the truism 'the absence of detection is not the detection of absence' is relevant in this case as for any assay. Also, the accuracy and reproducibility of this assay limits our ability to measure a moderate change in the reservoir size.

PCR is being extensively explored as an alternative to measure the reservoir, but relying on an approach based on the quantification of DNA also presents challenges. For example, viral RNA levels in the blood drop approximately four logs in the two months following ART initiation, whereas viral DNA levels in the blood drop only one log over a year. Therefore, we can certainly measure the presence of viral DNA, but we do not really know how to use DNA levels to evaluate the size of the reservoir, which is a tiny fraction of the total (largely defective) DNA signal.

Alternatively, quantifying the expression of viral RNA in latently infected cells after their activation is likely to be very useful, although this strategy also detects the expression of defective DNAs (that may not necessarily give rise to virion production). Full-length infectious genomes that can reseed active replication will be included in the induced RNA signal and the combined expression of defective and infectious DNA will increase the total signal, facilitating detection. However, at some point, we are probably going to need to use a measurement, such as the time to rebound after therapy discontinuation, as the most convincing evidence of a change in reservoir size, although this presents ethical and safety questions.

I also believe there is a place for measuring the complexity of the viral population in such studies (by sequence analysis), which could elucidate the size and sources of the reservoir; for example, a

longer time to rebound that reflects a smaller reservoir should also have reduced sequence complexity.

R.F.S. Most investigators consider the quantitative VOA (QVOA) to be the 'gold standard'. The value of this assay is that it quantitates the frequency of cells that produce replication-competent virus following a single round of T cell activation. This assay was used to demonstrate the presence and persistence of latently infected resting CD4⁺ T cells. As discussed below, the QVOA can be best thought of as a definitive minimal estimate of reservoir size. However, although the assay is highly reliable, it is also time consuming and expensive; therefore it is currently only carried out in a few research laboratories.

As an alternative, many investigators use PCR to detect HIV DNA. This approach is extremely problematic. For example, we have shown that the vast majority (approximately 98%) of HIV proviruses are highly defective and incapable of replication. This explains why PCR-based assays give infected cell frequencies that are much higher than, and poorly correlated with, the QVOA. Furthermore, PCR-based assays for HIV proviruses cannot be used as a surrogate for the measure of reservoir size in HIV cure studies, because the defective proviruses may respond differently to eradication strategies, compared with cells harbouring replication-competent proviruses.

A newer class of assays utilize a single round of T cell activation to induce HIV gene expression, followed by reverse transcription PCR (RT-PCR) analysis of intracellular HIV RNA or genomic viral RNA in virions released from infected cells. However, these assays may detect some defective proviruses that are still capable of HIV gene expression. Furthermore, these assays, similarly to the QVOA, rely on a single round of T cell activation, and we have recently demonstrated that this strategy fails to induce all of the proviruses that have the potential to become activated *in vivo*; some additional proviruses can be induced to produce replication-competent virus by additional rounds of T cell activation. The presence of these intact proviruses that are not induced in the first round of T cell activation further complicates reservoir measurement and suggests that future strategies should attempt to measure these proviruses. Thus, with current assays, we can bracket the size of the latent reservoir, but cannot accurately measure it.

D.M.M. This is a rapidly evolving area of the field, but many useful assays already exist. The current challenge is how to best interpret each assay. For example, measures of HIV DNA are of limited utility given the large excess of defective HIV DNA genomes. Measures of cell-associated viral RNA or virion RNA released from the cell are quite useful, although the relative values of many such assays recently developed requires further study. Nonetheless, although these RNA-based assays overestimate the number of cells that can express replication-competent HIV (as they can also detect RNA production from defective virus), they enable an assessment of latency-reversing agents (LRAs) seeking to disrupt proviral quiescence.

The QVOA is said to be a 'gold-standard' and is very useful. Currently, the QVOA provides a minimal estimate of the true latent reservoir, as the single round of *ex vivo* stimulation does not induce the expression of every replication-competent genome. However, this shortcoming may be improved by future modifications, including the introduction of several rounds of stimulation. Furthermore, although the QVOA is resource intensive, it is remarkably robust and reproducible over time when a patient is studied serially. In addition, the ability of the assay to reliably measure a reduction in the frequency of latent infection of 0.5 log or greater makes the QVOA a suitable measure for interventions that deplete persistent infection, as it is likely that extensive depletion of the latent reservoir is necessary to achieve clinical significance⁷.

Finally, assays are under development to detect the expression of viral antigen in rare, latently infected cells. Although many cells detected in such an assay will express only defective viral particles or antigens that are not a threat to the patient, such an antigen assay might be more accessible than the QVOA and might be used more broadly in future clinical settings.

M.J.C. Assays used to monitor the effects of cure strategies should not only measure viral activation by HIV RNA and virus production, but should also lead to the determination of the residual number of cells containing HIV genomes following treatment. This has proven to be problematic in the past, as infected individuals identified as having undetectable levels of HIV in the plasma have experienced viral rebound following prolonged ART cessation. Monitoring HIV in tissues is even more problematic, particularly in the CNS, where

the localized effects of virus activation are more likely to be detrimental to the infected individual and less able to be detected and treated.

There is currently no assay that accurately measures virus activation following treatment with LRAs in the CNS. Furthermore, it is unlikely that those levels of activation would result in detectable levels of virus in the CSF. However, CNS disease can occur in the absence of CSF viral load. For example, astrocytes in the brain have been demonstrated to be infected with HIV and, despite a non-productive infection, the frequency of astrocyte infection correlates with the severity of dementia⁸, which is thought to be associated with the production of Tat⁹. Notably, none of the current antiretrovirals prevents post-integration transcription, making the CNS possibly susceptible to untreatable damage owing to the activation of transcription of latently infected astrocytes. As there are currently only limited and somewhat unpredictable biomarkers that measure CNS activation and damage, it is very difficult to monitor the events that take place in this organ. Given the varied nature of potential HIV tissue reservoirs, at least at this time, it is difficult to envisage a 'gold standard' assay that can universally determine the effectiveness of cure strategies across the different reservoirs.

S.G.D. There are three potential readouts in HIV cure research: reservoir reduction, latency reversal and drug-free remission. Measuring the reservoir of replication-competent virus that is able to reignite replication is nearly impossible with current assays, for reasons others have delineated above. Indeed, even if we had a perfect way to measure a replication-competent virus population in the blood, I doubt this would be sufficient, as most of the cells harbouring such virus reside in lymphoid tissues that are difficult to access, and may not freely circulate in the blood. Our group favours direct measurement of virus-producing cells using radiolabelled tracers and imaging, and/or indirect measurements using virus-specific host responses, such as HIV antibodies, but our work on these approaches has only just begun.

Measuring latency reversal is far easier, as we can simply measure plasma viraemia, which is based on HIV RNA levels. The field is benefitting from the enormous decades-long investment in the development of this assay for standard antiretroviral drug development. In the context of potent and sustained ART, the level of viraemia is

barely detectable and often undetectable. Demonstrating a reduction in viraemia is typically not possible. Demonstrating an increase in viraemia during latency reversal, however, is straightforward, and several studies have already convincingly shown such increases.

Measuring a drug-free remission is theoretically straightforward. Interrupting therapy and monitoring plasma HIV RNA levels will provide direct assessment of this outcome. In practice, however, there are lots of issues, including the potential harm associated with resurgence in HIV replication. This harm could be mitigated by monitoring HIV RNA levels a few times a week, and resuming therapy once the virus becomes detectable, but this gets burdensome and expensive. Point-of-care viral load monitoring that can be done in the clinic or even at home would be hugely beneficial; such assays are being developed.

Q *One of the main goals of delineating the HIV reservoir is to potentiate the development of therapies that would enable its elimination, leading to the clearance of persistent HIV infection. Therefore, how do recent developments in elucidating the cellular and tissue components of the viral reservoir, including how they are established and how they contribute to disease reactivation, affect the cure efforts against HIV? In your opinion, what is the best strategy to eliminate latent infection, and what are the main challenges that must be overcome to achieve this goal?*

D.M.M. We are at the beginning of a challenging journey towards therapies that could induce a drug-free remission of HIV disease, and perhaps true cures. Efforts to develop LRAs were the first to begin and, more recently, approaches to clear persistent infection once latency has been reversed have gained momentum. This approach must be cautious and based on the best science and is poorly served by the simplified moniker of 'kick and kill'. Decades of work to understand the antiviral immune response and develop prophylactic vaccines will be directly translatable to the efforts to clear persistent infection (the so-called 'kill'). It will be challenging to develop effective LRAs, given the high bar for safety required in this healthy patient population, and the scientific challenges of targeting diverse viral populations governed by the same machinery that regulates many of the functions of uninfected cells. Furthermore, as latent infection in central memory T cells is targeted, other reservoirs

may prove to pose further barriers to cure. Although other approaches to HIV cure, such as genome editing, may eventually be accessible, the original 'kick and kill' concept has made significant progress, with the potential that further efforts may deliver treatments that could be effective and delivered across the world.

M.J.C. One of the most significant and challenging obstacles to HIV eradication continues to be the predicted existence of tissue reservoirs. Although it is acknowledged that these reservoirs are likely to exist and that large efforts are being made to identify and characterize them, the impact and nature of these reservoirs remains unclear. It is paramount that we clearly define the origin of HIV reservoirs in the plasma and also in the tissues.

One example of a potential tissue reservoir that can influence how we think about cure efforts is the CNS. For the CNS, there is a substantial amount of data to suggest that HIV persists within certain long-lived cells. However, the elimination of these infected cells in the CNS may not be a viable option for a cure, owing to the limited replenishing capacity of these cells. Nonetheless, these cells may have an important role during reactivation. Numerous studies have shown that HIV isolated from the CNS is distinct from that isolated from non-CNS tissues, and the virus in the CNS can also be uniquely regulated, thus altering the response of these tissues to activators that are currently being tested in clinical trials¹⁰. Furthermore, even if the CNS is established as a viable reservoir of HIV, it is unlikely that all infected patients will harbour a CNS reservoir. Therefore, it is necessary to understand who harbours a CNS reservoir and to determine its potential to replenish the viral pool in the periphery.

Should it be deemed necessary to address the CNS reservoir, how can this be achieved? This is problematic because ART has a varied effectiveness within the CNS. However, HIV isolated from the CNS has unique regulatory mechanisms with altered responsiveness to activators, which could be used to our advantage, should we opt for a functional cure over a sterilizing cure. For example, the use of activators with limited CNS penetration or with a documented reduced capacity to activate CNS-derived HIV could protect the CNS while enabling the activation of non-CNS reservoirs.

Given the current gaps in our knowledge, the major challenge in moving towards a cure is the clear identification and

characterization of all potential reservoirs of HIV; only then can we determine what is really achievable and how this can be achieved as we devise strategies for eradication.

R.S. It took the combination of three potent antiretroviral drugs given together, and over 10 years of drug development and clinical trials, to achieve suppressive therapy. Cure research will require even longer, as these efforts require targeting host pathways and our knowledge of the host always lags far behind our knowledge of the virus. Also, as with drug development, we need to be able to measure incremental success. We currently do not have assays that can reliably measure a twofold reduction in the reservoir, but achieving this would be a remarkable first step. Furthermore, we may not learn about smaller alternative reservoirs until we remove the resting T cell reservoir and see what grows out next. We are likely to learn about the efficacy of strategies that are designed to engineer the host in order to control virus released from the reservoir (such as therapeutic vaccines or other interventions) before we understand how to induce all latent proviruses or genetically inactivate them.

S.G.D. Given our experience with the Berlin Patient, who was cured of HIV following haematopoietic stem cell transplantation, this strategy (with or without gene modification) is certainly the most feasible way to cure someone. However, such approaches will never be applicable to a global epidemic.

With regard to drug therapy, the current paradigm is ‘kick and kill’, by which latency is reversed and virus producing cells are eliminated, leading, over time, to complete or near-complete eradication of the virus. Achieving such an outcome is unlikely with current strategies. We clearly need more powerful ‘kick’ agents and the hunt for possible ‘kill’ agents is just now beginning.

It only takes one replication-competent virus to ignite an explosion in virus replication. Therefore, our group is focused on what I prefer to call ‘reduce and control’, in which we use approaches to reduce the overall size of the reservoir while enhancing the capacity of the immune system to control the residual virus in a sustained manner. Experience with those rare individuals who naturally control HIV in the absence of therapy (‘elite controllers’) and perhaps those who are apparently able to durably control

their virus after short-term exposure to ART in early infection (‘post-treatment controllers’) suggests that we will need to achieve at least three outcomes before ART can be safely interrupted: a small reservoir size, low levels of immune activation and a sustained host-response that can control residual virus. A combination of ‘kick and kill’ strategies to reduce the reservoir with a vaccine that sustains an immune response may achieve these outcomes. This seems to be the most likely pathway to a curative intervention that is effective and scalable.

R.F.S. Many cure strategies are being pursued, but I believe that the most logical one is to directly target the latent reservoir in resting CD4⁺ T cells through a ‘kick and kill’ strategy. We know that this reservoir is a barrier to eradication in everyone with HIV infection. To eliminate it, we first need to turn on HIV gene expression in latently infected cells with LRAs. Otherwise, it is essentially impossible to distinguish infected cells from uninfected cells. This can be done safely in patients on ART. The antiretroviral drugs are so effective that we do not need to worry about new cells becoming infected. The main problem is finding effective LRAs. We have shown that LRAs must be evaluated in cells from patients because *in vitro* models do not accurately predict LRA activity. In addition, it is important to compare the activity of LRAs with a positive control — namely, agents that induce T cell activation — because T cell activation is the most effective way to reverse latency. Many LRAs have been described, but most have poor activity in patient cells compared with T cell activation. Recently, combinations of LRAs that are nearly as effective as T cell activation have been identified, but it is unclear whether they can be safely administered to patients.

Another problem is that reversal of latency is not sufficient. For example, we have shown that latently infected cells do not die following latency reversal. Therefore, we need to induce killing by immune effector mechanisms. We have found that HIV-specific cytolytic T cells (CTLs) from most patients on ART are relatively ineffective in killing infected cells after the reversal of latency, unless the CTLs are first stimulated by antigen. In addition, we have shown that unless ART is started early in the course of infection, the latent reservoir is composed almost entirely of proviruses with escape mutations in dominant CTL epitopes. Therefore, eradication strategies may need to include a therapeutic vaccination to

stimulate CTL specific for subdominant epitopes or some other intervention to promote the death of infected cells. We estimate that a two- to three- log reduction in the latent reservoir will be needed to enable a prolonged ART-free remission, but the possibility of a late rebound in viraemia will always be present unless all latently infected cells are eliminated.

Melissa J. Churchill is at the Centre for Biomedical Research, Burnet Institute, Melbourne, Victoria 3004, Australia.
churchil@burnet.edu.au

Steven G. Deeks is at the Department of Medicine, University of California, San Francisco, California 94110, USA.
steven.deeks@ucsf.edu

David M. Margolis is at the University of North Carolina (UNC) HIV Cure Center, Institute of Global Health and Infectious Diseases, and the Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA.
dmargo@med.unc.edu

Robert F. Siliciano is at the Department of Medicine and Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.
rsiliciano@jhmi.edu

Ronald Swanstrom is at the Lineberger Comprehensive Cancer Center, the Department of Biochemistry and Biophysics, and the University of North Carolina (UNC) Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA.
ron_swanstrom@med.unc.edu

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Competing interests statement
The authors declare no competing interests.