Establishment of latent HIV-1 reservoirs: what do we really know?

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Abstract

Despite our ability to suppress HIV-1 replication indefinitely in people on optimal combined antiretroviral therapy (cART), HIV-1 persists as a stably integrated and replication-competent provirus in a heterogeneous collection of long-lived cells (often referred to as 'latent reservoirs') in all individuals on treatment. Reactivation of these latent proviruses is believed to be responsible for the rebound viraemia that can be seen in nearly all people following treatment cessation. Hence, the persistence of HIV-1 in latent reservoirs remains one of the greatest challenges in current HIV cure research. Latent HIV-1 reservoirs are established early during the acute phase of the infection, possibly before the virus appears in the systemic circulation. As well as the issue of timing, we review the proposed hypotheses on the mechanisms by which this latent state is believed to be established early in the course of the infection and the effect of early initiation of cART on the size and stability of these reservoirs.

We conclude that prevention of the establishment of latent HIV-1 reservoirs by even extremely early initiation of cART proves to be practically impossible. However, early treatment initiation remains one of the crucial interventions needed to achieve the ultimate goal of a functional cure for HIV-1 infection because of its ability to reduce the overall size of HIV-1 reservoirs. Together with other interventions, early cART initiation may thus eventually lead to a state of better control over the residual amount of virus in the body, allowing people to stay off treatment for prolonged periods of time.

Introduction

The implementation of combination antiretroviral therapy (cART) has led to a dramatic improvement in outcomes for people living with HIV, turning a previously life-threatening illness into a chronic condition [1].

Despite its great success, it remains difficult to provide this revolutionary therapy to all people living with HIV, especially to those in resource-limited settings. This is particularly problematic given the need for strict therapeutic adherence. Taken together with the possibility of drug toxicity, drug–drug interactions, persistent immune dysfunction and ongoing low-grade inflammation in people on therapy, there is a growing interest in finding a cure for HIV rather than control of HIV replication [2]. The report of the Berlin patient, who is considered to be cured, and some cases of long-lasting remission have strongly fuelled the ongoing research in this field [3–6].

Indeed, cART is very effective in suppressing plasma virus levels to below the limit of detection of clinical commercial assays (20–50 copies HIV-1 RNA/mL), yet fails to be curative as viral loads rebound quickly upon cART cessation, typically within 2 weeks. This makes lifelong treatment an absolute necessity and the current standard of treatment for every individual living with HIV [7,8]. The main reason for viraemic rebound after cART cessation is believed to be the presence of stable reservoirs of HIV-1 provirus in resting CD4 T cells, even in patients on long-term cART [9–11].

The main focus of recent research has been towards eliminating viral reservoirs via a strategy called the 'shock and kill' [12]. Briefly, latent proviruses are reactivated with latency-reversing agents, restarting viral replication in the infected cell. This should lead to the elimination of these cells through viral cytopathic effects or by immune-mediated killing as they now express viral proteins. So far, attempts to purge the reservoir in this fashion have been largely unsuccessful, since both parts of the strategy, shock and kill, still need optimising [13]. At the same time, other creative strategies (e.g. 'block and lock', which aims to create a permanent nonproductive state of infection) are also being investigated [14].

In order to optimise these approaches and to create new strategies to target viral reservoirs of HIV-1, a more thorough understanding of their biology, composition, distribution and dynamics will be of the utmost importance. We review the evidence on the establishment of latent HIV-1 reservoirs during the acute phase of the infection.

Background

HIV reservoirs

The term 'latency' in virology usually refers to a nonproductive state of infection of a pool of cells that are transcriptionally silent, but retain the capacity to produce infectious virus particles upon stimulation [15]. This is different from 'clinical latency', a term formerly used to describe the clinically asymptomatic phase following the acute infection with HIV-1 and before the development of AIDS.

A reservoir is defined as 'a cell type or anatomical site in which a replication-competent form of virus accumulates and persists' [16], in spite of long periods of cART-suppressed viraemia [15]. Since various types of cells in different anatomical locations can fulfill these criteria, it is more accurate to consider HIV reservoirs in a plural form. Of note, this definition does not include most integrated HIV-1 genomes, as many are defective [17]. Evidence seems to indicate that even on suppressive cART there may be some ongoing active viral replication, in selected patients and/or in certain sanctuary sites [15]. These reservoirs may significantly contribute to the problem of viral persistence on therapy, but they do not qualify as 'latent' reservoirs in the stricter sense. As such, we will not discuss them in great detail.

It should also be noted that, since the focus of our review was on the timing of reservoir establishment, we did not perform a

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systematic review of the literature on cellular and anatomical reservoirs and sanctuary sites for HIV infection. When considering HIV infection, timing and location of reservoir formation cannot be separated, hence, we will provide a short overview of the current knowledge on cellular and anatomical HIV reservoirs (including possible sanctuary sites). For more extensive reviews we refer to [18–22].

Cellular reservoirs

HIV primarily infects cells of the immune system and in particular CD4 T cells. They also remain the best characterised reservoirs for HIV-1 in virally suppressed patients on cART [18–20]. T cells are classified according to their differentiation and memory status, or their effector functions, based on the expression of cell surface markers [18,19,23]. Naïve CD4 T cells are produced in the bone marrow and move to the thymus where they undergo antigenic selection. After stimulation, these cells proliferate and differentiate into active, specialised effector cells, including helper T cells (TH1, TH2, TH17 and TH9), follicular T helper cells (TFH) and regulatory T cells [18,23]. These activated CD4 T cells are the preferential targets for productive HIV-1 infection but they have short lifespans owing to cytopathic effects of the virus or cytotoxic T lymphocyte-mediated killing [24]. However, some effector cells can revert to a resting state and differentiate into long-lived memory CD4 T cells [25]. Different types of memory CD4 T cells exist : stem cell memory (TSCM), central memory (TCM), transitional memory (TTM), effector memory (TEM), migratory memory (TMM), tissue resident memory (TRM) and terminally differentiated (TTD) cells [18,19,23]. Studies have shown that the distinct CD4 memory T cell subsets are infected to a different extent by latent HIV-1 and may support viral persistence through various mechanisms [25,26]. Central memory CD4 T cells are still considered to be the most important reservoir of latent HIV [18,19]. Interestingly, a skewed distribution of HIV-1 reservoirs from the long-lived TCM compartment towards shorter-lived populations of TTM and TEM seems to be important for natural control of the virus, as can be seen in both 'elite-controllers' (EC) and in 'post-treatment controllers' (PTC) [6,27]. Early cART initiation seems to protect the frequency of infection of all memory T cell subsets [28–30], with one study showing a relative protection of the TCM subset [31], although another subsequent study failed to replicate these findings and instead found a higher contribution of memory stem cells and central memory T cells to the latent reservoir [30]. It has therefore not been consistently established whether the TCM subset would be more protected than others by early treatment. Even haematopoietic precursor cells may contribute to the latent reservoirs, although results are conflicting [32,33].

Other immune cells have been implicated as reservoirs as well, especially myeloid cells. Macrophages are a known target for HIV infection [34], but their role as a reservoir remains debated [18–22,35]. Macrophages are not killed by viral cytopathic effects *in vitro*, which would make them a likely reservoir [35,36]. However, studies on HIV infection and latency in macrophages have been complicated by the diversity of these cells. Monocyte-derived macrophages appear in tissues following a local injury and can be replenished from the blood compartment, whereas tissue resident macrophages are established during fetal development [37]. They are maintained independently and are highly specialised per organ (into Kupfer cells, alveolar macrophages, intestinal macrophages, microglia cells etc.) [37]. Each subset of TRMs has a different susceptibility to HIV infection. Macrophages in the gut-associated lymphoid tissue (GALT) appear to be quite resistant to infection, except in the rectum, whereas alveolar macrophages seem relatively permissive [38–41].

Dendritic cells are a heterogenous group of antigen-presenting cells, some with lymphoid and some with myeloid origin, with different features depending on their anatomical location [42]. They are susceptible to HIV infection *in vitro*, but appear less so than CD4 T cells *in vivo*. Still, dendritic cells are thought to aid the dissemination of HIV by sequestering intact virions and presenting them to T cells, thus infecting them [43]. Follicular dendritic cells in B follicles actually derive from perivascular precursors and may contribute to the reservoir in a different way: they may carry a stable pool of HIV-1 virions on their surface without being infected [44,45]. Finally, other cell types such as epithelial cells, fibrocytes and astrocytes are sometimes implicated as reservoirs, although these claims still need to be further investigated [19–22].

Anatomical HIV reservoirs

After HIV enters the body and local replication occurs at the site of entry, HIV quickly disseminates to the lymph nodes (within days) and later on (within weeks) to the bloodstream [46]. At this point, the virus spreads throughout the entire body as individual virions, by the transport of infected immune cells and by cell-to-cell transmission [20,47]. Indeed HIV-infected cells have been found in the brain (and cerebrospinal fluid), the lungs, kidneys, liver, adipose tissue, in the gastrointestinal tract, the male and female genitourinary systems and bone marrow [20,22]. However, the lymphoid tissues (spleen, thymus, lymph nodes, GALT) are the most important sites of viral replication during active infection, and HIV DNA can still be detected in the lymph nodes after years of cART [22]. Within these organs, HIV is usually found within T cells and macrophages, but also in more organ-specific cell types (such as epithelial cells, microglia, astrocytes and podocytes) [19,20,22]. The contribution of all these anatomical compartments as latent reservoirs is still debated.

Overall, research on HIV reservoirs is still in its infancy. There is much data from *in vitro*, *in vivo* and animal models but as yet no gold standard to determine the size of the latent reservoir. Patient populations are highly heterogeneous (virus strain, route of transmission, ethnicity, lifestyle, timing of cART, duration and type of treatment, rate of suppression, etc.). All of these factors may influence HIV reservoirs and confound experimental studies.

Methods

We conducted a systematic literature search of all English-language articles published between January 1997 and November 2017 using MEDLINE (PubMed). Where available, medical subject headings (MeSH) were used as search terms and included: *HIV-1* and *virus latency*. Additional search terms consisted of *reservoir, latent, persistence, establishment, generation, formation* and *seeding*.

A total of 301 articles was found using the above search terms. Of these, 79 relevant articles related to HIV-1 latency establishment were collected and reviewed, with no limitation of study design. Additional studies (commonly referenced and/or older and more recent articles of significance) were selected from bibliographies and references listed in the primary resources.

Results

Mechanisms of latent HIV-1 reservoir establishment

In general, when exploring the literature, there are two main models that can be distinguished to explain latent memory cell infection: pre-activation and post-activation latency.

In the pre-activation model of latency, resting CD4 memory T cells are directly infected by HIV-1 before they are re-activated by stimuli from the environment. This process of direct resting cell infection is usually considered very inefficient due to blocks at various points in the viral life cycle [48,49]. It therefore resolves mostly in non-integrated linear and cytoplasmic forms of the viral genome with a half-life of approximately 1–6 days, due to instability of the pre-integration complex (also called 'pre-integration latency') [50–52]. However, it has been shown that certain cytokines and chemokines from the tissue microenvironment have the ability to make these resting CD4 T cells more permissive to HIV-1 infection, resulting in integrated proviruses without inducing cell proliferation or upregulation of T cell activation markers *in vitro* [53–55]. It cannot, therefore, be entirely excluded that such mechanisms could also occur *in vivo*, as it has been previously shown that mucosal CD4 resting T cells can at least be productively infected by HIV-1 *in vivo* [56,57].

Alternatively, the post-activation model for latency establishment is derived from studying normal T cell biology. As previously described, a small subset of antigen-stimulated active CD4 T cells will return to a resting state, awaiting activation upon re-encounter of the same antigen in the future, hence fulfilling their memory potential [58]. In theory, activated CD4 T cells can become infected with HIV-1 in the process of transitioning back to this resting state, allowing integration of the proviral genome in a host cell lacking favorable conditions for optimal viral gene expression, hence escaping rapid destruction of the infected T cell [10,52,59]. Evidence in support of this theory has come indirectly from *in vitro* models showing that HIV-1 is able to establish a latent infection in actively replicating T cells which, after being cultured with specific cytokines that induce a resting cell phenotype, are capable of producing active virus after stimulation with latency-reversing agents [54,60,61].

In the latter view, latency is considered as being merely an epiphenomenon of the activation status of the cell. Recent studies, however, show that T cell activation status and HIV-1 latency might not be as tightly coupled as previously thought. In this respect, Calvanez *et al*. have repeatedly shown that HIV-1 latency can be established directly in both activated and resting CD4 T cells, but with a different ratio of productive versus latent infection in both cell types [54,62]. This view gives rise to yet another possible model, whereby HIV-1 latency established in activated CD4 T cells shortly after entry confers a greater potential to survive and possibly return to a resting memory state, contributing to the long-lived latent reservoirs [54]. HIV-1 integration-site selection may play a role in the mechanisms of establishment of HIV-1 latency in these activated CD4 T cells. Indeed HIV-1 integration is not random but targeted towards active genes [63–67]. Lens epithelium-derived growth factor (LEDGF/p75) is the main cellular co-factor of HIV-1 integration (reviewed in [68]). As a chromatin reader and transcriptional co-activator, LEDGF/p75 recognises nucleosomes associated with actively transcribed genes thereby tethering integrase to active chromatin [69–71]. Our lab developed small-molecule inhibitors of the LEDGF/p75-integrase interaction, called LEDGINs [72,73]. We also showed that these LEDGINs inhibit viral replication and integration [72,74–80] and also retarget residual integration sites away from active genes, making the residually integrated provirus more resistant to reactivation (and thus more latent) [80]. Another possible explanation for latency can be found in the theory of stochastic gene expression. According to this theory, it is believed that stochastic (or random) mutations in the critical HIV-1 transcriptional activator molecule (Tat) can hinder active HIV-1 transcription, influencing the latency decision independent of the target-cell activation state [81,82]. This could offer a plausible explanation for the fact that there remains a small but significant pool of replication-competent HIV-1 provirus in resting CD4 T cells that cannot be induced by the known 'deterministic' host cell-associated factors that influence its transcription process [83]. In addition, a relatively recent *in vitro* study has shown that removing activation stimuli from the environment of primary CD4 T cells infected with HIV-1 predictably lowers the cellular activation state, but surprisingly leaves the viral transcription activity unaffected [84]. In the same study, the investigators showed, via the development of a synthetic circuit, that the Tat positive feedback is sufficient to control viral transcription without the need for cellular activation. These data are in support of previous findings that the establishment of latency can both be prevented and reversed by sufficient levels of intracellular HIV-1 Tat [85].

These different proposed models of HIV-1 latency establishment may not necessarily be mutually exclusive, but could also be complementary. Most studies on this topic are *in vitro*, leaving many questions about the extent to which, or if, these different mechanisms contribute to this complex picture *in vivo*.

Time of seeding of latent HIV-1 reservoirs

The discovery of long-lived stable cellular reservoirs for HIV-1 has led to a pessimistic outlook on strategies aimed at eradicating residual latent HIV-1 with cART alone [11,86]. However, initiating cART before the establishment of latent HIV-1 reservoirs can prevent lifelong acquisition of HIV, as this is the principle behind post-exposure prophylaxis (PEP). This brings us to the vital question of how early reservoirs are seeded.

The easy answer to this question would be 'very early in the course of the infection', at least before the symptomatic primary infection phase occurs and the available commercial diagnostic assays can confirm the diagnosis [50,87]. The initiation of cART as early as 10 days after the presentation of symptoms of a primary HIV-1 infection could not prevent the generation of latently infected resting CD4 T cells carrying replication-competent provirus [87]. Even more recently, a study investigating the effect of treatment interruption on eight participants who acquired HIV-1 and started cART in Fiebig stage I (corresponding to the first 2 weeks of infection, Table 1) found that there was rapid viral rebound in all individuals who had a median of 2.8 years of treatment [88]. Moreover, it was previously shown in rhesus monkeys that very early initiation of cART on day 3 after intrarectal SIV infection and before systemic viraemia occurred, could not prevent viral rebound after 24 weeks of treatment [89]. Although not in humans, this study reflects the strikingly early seeding of viral reservoirs during the 'eclipse phase', when the infection is still limited to the mucosa and local lymphoid tissue.

In most documented cases of children with perinatally acquired HIV-1 where cART is initiated within 30 hours of delivery, rebound viraemia occurs at some point after cART discontinuation. This seems to suggest indirectly that such an early seeding of viral reservoirs as seen in rhesus monkeys could also occur in humans [90–92]. However, the precise moment of infection could not be defined in these children and could have occurred *in utero* sometime before delivery.

It is particularly challenging to study the precise time at which reservoirs are established in humans because we cannot reliably detect the virus this early in the infection because our methods of detection mainly focus on the systemic viraemia phase. However, the possibility of the viral reservoirs being seeded during the mucosal eclipse phase of the infection calls for a better understanding of the early events after transmission of the virus. Much

Table 1. The Fiebig stage of primary HIV infection based on the emergence of a specific set of viral markers in the plasma of individuals who have acquired HIV. Classifications are based on a parametric Markov model

Fiebig stage	Cumulative duration		HIV RNA	P24 antigen	Antibody (EIA)		Western blot
	Days	95% confidence interval			NS	S	
I	5.0	3.1–8.1	+				
II	10.3	7.1–13.5	+	+			
III	13.5	10.0–17.0	+	+		+	
IV	19.1	15.3–22.9	+	+		+	Indeterminate
V	88.6	47.4–129.8	+	+	+	+	+ (p31 band negative)
VI	Open ended		+	+	+	+	+ (p31 band positive)

EIA: enzyme immunoassay: NS: not sensitive (refers to second generation not IgM-sensitive immunoassay); S: sensitive (refers to second generation IgM-sensitive immunoassay).

of our current knowledge on this topic, comes from studies with a rhesus macaque model of SIV vaginal transmission by Haase *et al.* [57].

In these studies, it is shown that there is an eclipse phase of 1–3 days post-infection in which SIV DNA is detectable in the tissues at the portal site of entry before systemic viraemia occurs, with only few cells producing viral RNA representing progeny virus [93]. During these early stages of the infection, it appears that mostly resting CD4 T cells are productively infected by SIV at the portal site of entry. The same findings were observed in lymph node and tonsillar biopsies performed during the acute stage of HIV-1 infection in humans [56].

It is reasoned that because resting CD4 T cells greatly outnumber other potential target cells at the portal site of entry (here, the vaginal mucosa tissue) there is a greater possibility of virus encountering and infecting a resting cell until immune activation causes an influx of activated CD4 target cells to which the infection is later preferentially disseminated [94].

A more thorough characterisation of the activation state of these T cells productively infected by both SIV and HIV-1 in the early stage of the infection was performed by testing them for the activation markers CD25, CD71, CD30, CD38, CD134 and Ki67. Results showed that although cells with and without these activation markers were present, most cells were negative for each marker and could be categorised as resting [56].

Only a small fraction of the resting CD4 T cell population is infected during the first week after initial acquisition and a nearly 20-fold decrease in SIV RNA+ Ki67- cells occurs between week 1 and 4 [94]. These findings suggest that an as yet undefined subpopulation of resting cells – possibly those in a slightly higher state of activation – can support productive infection. These cells are then either eliminated by viral cytopathic or host immune-effector mechanisms or can be activated. Alternatively, this decrease may reflect a conversion of the pool of initially productively infected cells to a state of latent infection [94]. Although it is very hard to provide direct evidence for these theories in humans, these studies show that the initial environment during the mucosal eclipse phase of the infection after sexual transmission of HIV-1, could potentially support the generation of a pool of latently infected resting CD4 T cells as early as a few days after initial acquisition. Whether these potentially latently infected cells have the ability to persist during the lifetime of the individual has not been clearly established.

The effect of timing of antiretroviral therapy initiation on size and stability of HIV-1 reservoirs

In spite of the previously described evidence for very early seeding of viral reservoirs during HIV infection, numerous studies have focused on the effect of early cART initiation on the size of the reservoirs and the possibility of achieving HIV remission when treatment is interrupted (post-treatment control).

It has been repeatedly shown that patients who start cART early during the acute HIV-1 infection phase (up to 6 months after acquisition) exhibit a lower overall reservoir size compared to those who start treatment later, for example during chronic infection [29,30,95–103]. Although these studies differ in patient characteristics, timing of cART initiation, total treatment duration and assays for reservoir size measurement, they all show a significant decrease in total HIV burden when cART is started early. It does appear that the earlier treatment is initiated during acute HIV-1 infection (AHI), the smaller the size of viral reservoirs after viral suppression, suggesting an escalation of HIV-1 reservoir seeding over time [96,100]. Moreover, Ananworanich *et al.* recently showed how during the acute stage of the infection in participants who received no treatment, total HIV-1 DNA in PBMCs peaked 2 weeks after enrollment, reaching a set point 2 weeks later with little change thereafter, while integrated HIV-1 DNA seemed to increase during untreated infection [101]. Very early cART could reduce the frequency of HIV-1 DNA-positive cells significantly, but this effect is probably less pronounced if treatment is initiated in later stages of the infection [100].

Even when cART is started during the acute infection phase of the disease, no further decay of the frequency of cells harbouring replication-competent HIV-1 to less than 0.5 cells per million could be achieved after long-term treatment, suggesting a subset of very early latently infected cells may persist indefinitely [97].

Starting treatment early may, however, be clinically relevant since high HIV-1 DNA levels are an independent predictor of disease progression [104,105] and pre-treatment total HIV-1 DNA and cell-associated HIV-1 RNA levels predicted time to viral rebound upon cART interruption in several studies [105,106]. Additionally, the inability to detect HIV-1 DNA does not seem to exclude the possibility of rebound plasma viraemia occurring at some point following treatment interruption [4,90,107].

Since lower overall reservoir size has been associated with increased time to viral rebound and early cART reduces the size of viral reservoirs, it has been hypothesised that early cART initiation

during AHI would delay viral rebound following treatment interruption. Yet cART initiated as early as Fiebig stage I (HIV-1 RNA+, p24-, HIV-1 IgM-; Table 1) did not result in a significantly longer time to viral rebound after treatment interruption when compared to cohorts with chronic HIV-1 infection. This suggests that early cART alone is not sufficient to induce HIV-1 remission and additional strategies to eliminate or control latently infected cells are required [88]. In contrast, long-term virological remission after interruption of cART has been achieved in three children with perinatally acquired HIV-1 who started treatment early [5,108,109], and several adults who started treatment during primary infection [6]. These cases do suggest that as well as the size of the reservoirs containing replication-competent proviruses, other factors such as the absence of ongoing replication and HIV-1 specific immunity are also of great importance in achieving durable HIV-1 remission [110].

Discussion

The focus of HIV research has shifted from 'care' to 'cure' with the aim of finding alternatives for the current standard of treatment: chronic suppressive antiretroviral therapy for the lifetime of the individual living with HIV [2].

Two distinct interpretations of a possible cure have been introduced. In the first, the ultimate goal remains the absolute and complete eradication of the virus from the body of individuals leading to permanent virological remission off treatment, an approach often referred to as a 'sterilising cure' [111]. It is believed that the Berlin patient was successfully cured using this approach. This case shows the great challenges that go with this strategy and raises questions concerning the safety, cost, feasibility and scalability of this approach [3]. This, combined with the inability to replicate this success story, has led to the second interpretation for cure, the aspiration for achieving a state of 'sustained virologic remission' or 'functional cure' in which individuals living with HIV can remain without treatment despite the objective persistence of replication-competent proviruses without rebound of plasma viraemia [8]. As discussed above, early initiation of cART is likely to be one of the crucial steps in achieving long-lasting remission without treatment and the containment of the establishment of the viral reservoirs during the early stages of the infection appears to be a major contributing factor. Yet, some important questions remain unanswered.

How early does treatment need to be started? The findings of Buzon *et al.* suggest that initiating treatment as early as 6 months might not suffice [30]. Most evidence suggests that the earlier treatment is started, the better [100,101]. Using the Fiebig classification for AHI to define the point of treatment initiation [112], treatment during the earliest stages of the infection (e.g. Fiebig I and II) logically offers the most likely chance for an eventual stable HIV remission (Table 1). However, the exact window of opportunity for treatment initiation to achieve this goal remains to be elucidated. Most of the post-treatment controllers of the VISCONTI cohort [6] were treated in the later stages of AHI (Fiebig IV and V), highlighting the importance of additional parameters, such as immune control, that should be considered when plans for treatment interruption are being made. Thus, an accurate AHI staging system based upon a thorough characterisation of patients at time of treatment initiation (including factors such as HIV reservoir size, HIV specific immunity markers, HLA profile) to identify those more likely to benefit from HIV cure efforts is still lacking in the field.

Currently, as most widely available diagnostic assays are antibody-dependent and based on showing seroconversion, with most tests returning positive results 4–6 weeks after infection, there is also the practical problem of identifying individuals as early as a few days after infection. Very early detection of HIV-1 infection is possible using nucleic acid testing (NAT), directly identifying viral genetic material in the systemic circulation [113]. The use of these assays is limited owing to their cost, availability and the stigma or lack of awareness of risk factors that prevents individuals from seeking early testing. Additionally, symptoms of the acute retroviral syndrome do not occur in every individual and since symptoms are very non-specific, individuals do not always recognise them as signs of possible HIV acquisition [114].

Finally, the length of treatment required before interruption is defensible is unknown and we do not yet have the tools to determine the appropriate time for safe treatment interruption. For example, Chun *et al.* published a case report showing viral rebound 50 days after cART discontinuation in a person who initiated therapy during acute infection and remained on treatment for 10.5 years, despite undetectable levels of HIV-1 DNA in peripheral blood CD4 T cells or in sigmoid colon biopsies at the moment of treatment interruption [107]. Similarly, in the cases of the Mississippi child [5] and the Boston patients [4], investigators also failed to detect HIV-1 DNA, yet rebound viraemia eventually occurred in all of the individuals.

A small HIV-1 reservoir does seem to be important for achieving post-treatment viral control as a reduced reservoir size is a common finding in those PTC and EC identified to date. A critical shortcoming that remains, however, is a gold-standard method to measure intact replication-competent proviruses [115]. The current gold standard for measuring this form of the viral reservoir, the viral outgrowth assay (VOA), appears to underestimate the amount of replication-competent proviruses by 60-fold compared to direct detection methods of intact proviruses by sequence analysis [83]. There is a growing interest in finding more accurate detection methods [116]. Recent innovations include the Tat/rev induced limiting dilution assay (TILDA) [117] and the use of a reporter cell-based assay based upon the TZM-bl cell line [118], both of which have increased sensitivity, require less blood volume, are faster, less labour intensive and less expensive compared to the VOA. A noteworthy improvement to the PCR-based strategies is the development of the intact proviral DNA assay (IPDA) [119,120]. This digital droplet PCR assay detects intact, cell-associated, full-length genomic HIV DNA with increased sensitivity. Further validation, however, is needed to confirm the applicability of these assays in cART interruption studies.

A recent study by Henrich *et al.* reports the case of two extremely early HIV-1 diagnoses and subsequent treatment initiations, namely at the end of the eclipse phase and at the beginning of the start of Fiebig I, preceding the acute infection phase [121]. Despite the complete loss of detectable HIV-1 in blood, colorectal and ileal tissue, an excised inguinal lymph node, PBMCs, cerebrospinal fluid and a bone marrow biopsy in one individual following 32 weeks of continuous cART, rebound viraemia occurred 225 days following treatment interruption. This study highlights the extremely early seeding of HIV-1 reservoirs in humans, making future efforts aimed at the prevention of the establishment of viral reservoirs very likely to fail. On the other hand, it also provides an affirmation that increased time to viral rebound can be seen in those starting treatment very early. Future studies aimed at achieving a prolonged HIV remission state are therefore more likely to be successful if they focus on a combination strategy including early cART initiation, prolonged treatment duration, eliminating or silencing infected CD4 T cells and increasing the anti-HIV immune response [111,122].

Acknowledgements

Declaration of interests

The authors declare no conflict of interest.

References

1. Deeks SG, Lewin SR, Havlir DV. The end of aids: hiv infection as a chronic disease. *Lancet* 2013; **382**: 1525–1533.
2. Deeks SG, Lewin SR, Ross AL *et al.* International AIDS Society global scientific strategy: towards an HIV cure 2016. *Nat Med* 2016 **22**: 839–850.
3. Hütter G, Nowak D, Mossner M *et al.*Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 2009 **360**: 692–698.
4. Henrich T J, Hu Z, Li JZ *et al.* Long-term reduction in peripheral blood hiv type 1 reservoirs following reduced-intensity conditioning allogeneic stem cell transplantation. *J Infect Dis* 2013: **207**: 1694–1702.
5. Persaud D, Gay H, Ziemniak C *et al.* Absence of detectable HIV-1 viremia after treatment cessation in an infant. *N Engl J Med* 2013: **369**: 1828–1835.
6. Sáez-Cirión A, Bacchus C, Hocqueloux L *et al.* Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. *PLoS Pathog* 2013: **9**: e1003211.
7. Davey RT, Bhat N, Yoder C *et al.* HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc Natl Acad Sci U S A* 1999: **96**: 1–6.
8. Richman DD, Margolis DM, Delaney M *et al.* Challenge of finding a cure for HIV infection. *Science* 2009: **323**: 1304–1307.
9. Chun T W, Stuyver L, Mizell S B *et al.* Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci U S A* 1997: **94**: 13193–13197.
10. Finzi D, Hermankova M, Pierson T *et al.* Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997: **278**: 1295–1300.
11. Siliciano JD, Kajdas J, Finzi D *et al.* Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T Cells. *Nat Med* 2003: **9**: 727–728.
12. Archin NM, Liberty AL, Kashuba AD *et al.* Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 2012; **487**: 482.
13. Thorlund K, Horwitz MS, Fife BT *et al.* Landscape review of current hiv 'kick and kill' cure research - some kicking, not enough killing. *BMC Infect Dis* 2017: **17**: 595.
14. Darcis G, Van Driessche B, Van Lint C. Review HIV latency: should we shock or lock? *Trends Immunol* 2017; **38** (**3**): 217–228.
15. Eisele E, Siliciano RF. Redefining the viral reservoirs that prevent HIV-1 eradication. *Immunity* 2012: **37**: 377–388.
16. Blankson JN, Persaud D, Siliciano RF. The challenge of viral reservoirs in hiv-1 infection. *Annu Rev Med* 2002: **53**: 557–593.
17. Chun TW, Carruth L, Finzi D *et al.* Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997; **387**: 183–188.
18. Kulpa DA, Chomont N. HIV Persistence in the setting of antiretroviral therapy: when, where and how does HIV hide? *J Virus Erad* 2015; **1**: 59–66.
19. Barton K, Winckelmann A, Palmer S. HIV-1 reservoirs during suppressive therapy. *Trends Microbiol* 2016; **24**: 345–355.
20. Mzingwane ML, Tiemessen CT. Mechanisms of HIV persistence in HIV reservoirs. *Rev Med Virol* 2017; **27**: 1–12.
21. Kandathil AJ, Sugawara S, Balagopal A. Are T cells the only HIV-1 reservoir. *Retrovirology* 2016; **13**: 86.
22. Wong JK, Yukl SA. Tissue reservoirs of HIV. *Curr Opin HIV AIDS* 2016; **11**: 362–370.
23. Chang JT, Wherry EJ, Goldrath AW. Molecular regulation of effector and memory T cell differentiation. *Nat Immunol* 2014; **15**: 1104–1115.
24. Coffin J, Swanstrom R. HIV pathogenesis: dynamics and genetics of viral populations and infected cells. *Cold Spring Harb Perspect Med* 2013; **3**: a012526.
25. Chomont N, El-Far M, Ancuta P *et al.* HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* 2009; **15**: 893–900.
26. Brenchley JM, Hill BJ, Ambrozak DR *et al.* T-cell subsets that harbor human immunodeficiency virus (HIV) *in vivo*: implications for HIV pathogenesis. *J Virol* 2004; **78**: 1160–1168.
27. Descours B, Avettand-Fenoel V, Blanc C *et al.* Immune responses driven by protective human leukocyte antigen alleles from long-term nonprogressors are associated with low HIV reservoir in central memory CD4 T cells. *Clin Infect Dis* 2012; **54**: 1495–1503.
28. Bacchus C, Cheret A, Avettand-Fenoel V *et al.* A single HIV-1 cluster and a skewed immune homeostasis drive the early spread of HIV among resting CD4+ cell subsets within one month post-infection. *PLoS One* 2013; **8**: e64219.
29. Josefsson L, von Stockenstrom S, Faria NR *et al.* The HIV-1 reservoir in eight patients on long-term suppressive antiretroviral therapy is stable with few genetic changes over time. *Proc Natl Acad Sci U S A* 2013; **110**: E4987–E4996.
30. Buzon MJ, Martin-Gayo E, Pereyra F *et al.* Long-term antiretroviral treatment initiated at primary HIV-1 infection affects the size, composition, and decay kinetics of the reservoir of HIV-1-infected CD4 T cells. *J Virol* 2014; **88**: 10056–10065.
31. Cheret A, Bacchus-Souffan C, Avettand-Fenoel V *et al.* Combined ART started during acute HIV infection protects central memory CD4+ T cells and can induce remission. *J Antimicrob Chemother* 2015; **70**: 2108–2120.
32. Josefsson L, Eriksson S, Sinclair E *et al.* Hematopoietic precursor cells isolated from patients on longterm suppressive HIV therapy did not contain HIV-1 DNA. *J Infect Dis* 2012; **206**: 28–34.
33. Durand CM, Ghiaur G, Siliciano JD *et al.* HIV-1 DNA is detected in bone marrow populations containing CD4+ T cells but is not found in purified CD34+ hematopoietic progenitor cells in most patients on antiretroviral therapy. *J Infect Dis* 2012; **205**: 1014–1018.
34. Gartner S, Markovits P, Markovitz DM *et al.* The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* 1986; **233**: 215–219.
35. Stevenson M. Role of myeloid cells in HIV-1-host interplay. *J Neurovirol* 2015; **21**: 242–248.
36. Swingler S, Mann AM, Zhou J *et al.* Apoptotic killing of HIV-1-infected macrophages is subverted by the viral envelope glycoprotein. *PLoS Pathog* 2007; **3**: 1281–1290.
37. Haldar M and Murphy KM. Origin, development, and homeostasis of tissue-resident macrophages. *Immunol Rev* 2014; **262**: 25–35.
38. King DF, Siddiqui AA, Buffa V *et al.* Mucosal tissue tropism and dissemination of HIV-1 subtype B acute envelope-expressing chimeric virus. *J Virol* 2013; **87**: 890–899.
39. McElrath MJ, Smythe K, Randolph-Habecker J *et al.* Comprehensive assessment of HIV target cells in the distal human gut suggests increasing HIV susceptibility toward the anus. *J Acquir Immune Defic Syndr* 2013; **63**: 263–271.
40. Jambo KC, Banda DH, Kankwatira AM *et al.* Small alveolar macrophages are infected preferentially by HIV and exhibit impaired phagocytic function. *Mucosal Immunol* 2014; **7**: 1116–1126.
41. Shen R, Meng G, Ochsenbauer C *et al.* Stromal down-regulation of macrophage CD4/CCR5 expression and NF-kappaB activation mediates HIV-1 non-permissiveness in intestinal macrophages. *PLoS Pathog* 2011; **7**: e1002060.
42. Banchereau J, Briere F, Caux C *et al.* Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; **18**: 767–811.
43. McDonald D, Wu L, Bohks SM *et al.* Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science* 2003; **300**: 1295–1297.
44. van Nierop K, de Groot C. Human follicular dendritic cells: function, origin and development. *Semin Immunol* 2002; **14**: 251–257
45. Spiegel H, Herbst H, Niedobitek G *et al.* Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T-helper cells. *Am J Pathol* 1992; **140**: 15–22.
46. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 Infection. *N Engl J Med* 2011; **364**: 1943–1954.
47. Kahn JO, Walker BD. Acute human immunodeficiency virus type 1 infection. *N Engl J Med* 1998; **339**: 33–39.
48. Zhou Y, Zhang H, Siliciano JD, Siliciano RF. Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4+ T cells. *J Virol* 2005; **79**: 2199–2210.
49. Blankson JN, Finzi D, Pierson TC *et al.* Biphasic decay of latently infected CD4+ T cells in acute human immunodeficiency virus type 1 infection. *J Infect Dis* 2000; **182**: 1636–1642.
50. Marsden MD, Zack JA. Establishment and maintenance of HIV latency: model systems and opportunities for intervention. *Future Virol* 2010; **5**: 97–109.
51. Pierson T, McArthur J, Siliciano RF. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. *Annu Rev Immunol* 2000; **18**: 665–708.
52. Chun TW, Finzi D, Margolick J *et al.* In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat Med* 1995; **1**: 1284–1290.
53. Saleh S, Solomon A, Wightman F *et al.* CCR7 ligands CCL19 and CCL21 increase permissiveness of resting memory CD4+ T cells to HIV-1 infection: a novel model of HIV-1 latency. *Blood* 2007; **110**: 4161–4164.
54. Chavez L, Calvanese V, Verdin E. HIV latency is established directly and early in both resting and activated primary CD4 T cells. *PLoS Pathog* 2015; **11**: e1004955.
55. Swiggard WJ, Baytop C, Yu JJ *et al.* Human immunodeficiency virus type 1 can establish latent infection in resting CD4+ T cells in the absence of activating stimuli. *J Virol* 2005; **79**: 14179–14188.
56. Zhang L, Chung C, Hu BS. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T Cells. *J Clin Invest* 2000, **106**, 839–845.
57. Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. *Annu Rev Med* 2011; **62**: 127–139.
58. Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* 2014; **14**: 24–35.
59. Chun TW, Carruth L, Finzi D *et al.* Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997; **387**: 183–188.
60. Bosque A, Planelles V. Induction of HIV-1 latency and reactivation in primary memory CD4+ T cells. *Blood* 2009; **113**: 58–65.
61. Jordan A, Bisgrove D, Verdin E. HIV reproducibly establishes a latent infection after acute infection of T cells in vitro. *EMBO J* 2003; **22**: 1868–1877.
62. Calvanese V, Chavez L, Laurent T *et al.* Dual-color HIV reporters trace a population of latently infected cells and enable their purification. *Virology* 2013; **446**: 283–292.
63. Schroder AR, Shinn P, Chen H *et al.* HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 2002; **110**: 521–529.
64. Jordan A, Defechereux P, Verdin E. The site of HIV-1 integration in the human genome determines basal transcriptional activity and response to Tat transactivation. *EMBO J* 2001; **20**: 1726–1738.
65. Maxfield LF, Fraize CD, Coffin JM. Relationship between retroviral DNA-integration-site selection and host cell transcription. *Proc Natl Acad Sci U S A* 2005; **102**: 1436–1441
66. Han Y, Lin YB, An W *et al.* Orientation-dependent regulation of integrated HIV-1 expression by host gene transcriptional readthrough. *Cell Host Microbe* 2008; **4**: 134–146.
67. Felice B, Cattoglio C, Cittaro D *et al.* Transcription factor binding sites are genetic determinants of retroviral integration in the human genome. *PLoS One* 2009; **4**: e4571.
68. Debyser Z, Christ F, De Rijck J, Gijsbers R. Host factors for retroviral integration site selection. *Trends Biochem Sci* 2015; **40**: 108–116.
69. van Nuland R, van Schaik FM, Simonis M *et al.* Nucleosomal DNA binding drives the recognition of H3K36-methylated nucleosomes by the PSIP1-PWWP domain. *Epigenetics Chromatin* 2013; **6**: 12.
70. Eidahl JO, Crowe BL, North JA *et al.* Structural basis for high-affinity binding of LEDGF PWWP to mononucleosomes. *Nucleic Acids Res* 2013; **41**: 3924–3936.
71. Ciuffi A, Llano M, Poeschla E *et al.* A role for LEDGF/p75 in targeting HIV DNA integration. *Nat Med* 2005; **11**: 1287–1289.
72. Christ F, Voet A, Marchand A *et al.* Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. *Nat Chem Biol* 2010; **6**: 442–448.

73. Demeulemeester J, Chaltin P, Marchand A *et al.* LEDGINs, non-catalytic site inhibitors of HIV-1 integrase: a patent review (2006–2014). *Expert Opin Ther Pat* 2014; **24**: 609–632.
74. Christ F, Shaw S, Demeulemeester J *et al.* Small-molecule inhibitors of the LEDGF/p75 binding site of integrase block HIV replication and modulate integrase multimerization. *Antimicrob Agents Chemother* 2012; **56**: 4365–4374.
75. Kessl JJ, Jena N, Koh Y *et al.* Multimode, cooperative mechanism of action of allosteric HIV-1 integrase inhibitors. *J Biol Chem* 2012; **287**: 16801–16811.
76. Balakrishnan M, Yant SR, Tsai L *et al.* Non-catalytic site HIV-1 integrase inhibitors disrupt core maturation and induce a reverse transcription block in target cells. *PLoS One* 2013; **8**: e74163.
77. Desimmie BA, Schrijvers R, Demeulemeester J *et al.* LEDGINs inhibit late stage HIV-1 replication by modulating integrase multimerization in the virions. *Retrovirology* 2013; **10**: 57.
78. Jurado KA, Wang H, Slaughter A *et al.* Allosteric integrase inhibitor potency is determined through the inhibition of HIV-1 particle maturation. *Proc Natl Acad Sci U S A* 2013; **110**: 8690–8695.
79. Le Rouzic E, Bonnard D, Chasset S *et al.* Dual inhibition of HIV-1 replication by integrase-LEDGF allosteric inhibitors is predominant at the post-integration stage. *Retrovirology* 2013; **10**: 144.
80. Vranckx LS, Demeulemeester J, Saleh S *et al.* LEDGIN-mediated inhibition of integrase-LEDGF/p75 interaction reduces reactivation of residual latent HIV. *EBioMed* 2016; **8**: 248–264.
81. Weinberger LS, Burnett JC, Toettcher JE *et al.* Stochastic gene expression in a lentiviral positive-feedback loop: HIV-1 Tat fluctuations drive phenotypic diversity. *Cell* 2005; **122**: 169–182.
82. Weinberger AD, Weinberger LS. Stochastic fate selection in HIV-infected patients. *Cell* 2013; **155**: 497–499.
83. Ho YC, Shan L, Hosmane NN *et al.* Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 2013; **155**: 540–551.
84. Razooky BS, Pai A, Aull K *et al.* A hardwired HIV latency program. *Cell* 2015; **160**: 990–1001.
85. Donahue DA, Kuhl BD, Sloan RD, Wainberg MA. The viral protein Tat can inhibit the establishment of HIV-1 latency. *J Virol* 2012; **86**: 3253–3263.
86. Finzi D, Blankson J, Siliciano JD *et al.* Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* 1999; **5**: 512–517.
87. Chun TW, Engel D, Berrey MM *et al.* Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. *Proc Natl Acad Sci U S A* 1998; **95**: 8869–8873.
88. Colby DJ, Trautmann L, Pinyakorn S *et al.* Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. *Nat Med* 2018; **24**: 923–926.
89. Whitney JB, Hill AL, Sanisetty S *et al.* Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature* 2014; **512**: 74–77.
90. Luzuriaga K, Gay H, Ziemniak C *et al.* Viremic relapse after HIV-1 remission in a perinatally infected child. *N Engl J Med* 2015; **372**: 786–788.
91. Bitnun A, Samson L, Chun TW *et al.* Early initiation of combination antiretroviral therapy in HIV-1-infected newborns can achieve sustained virologic suppression with low frequency of CD4+ T cells carrying HIV in peripheral blood. *Clin Infect Dis* 2014; **59**: 1012–1019.
92. Giacomet V, Trabattoni D, Zanchetta N *et al.* No cure of HIV infection in a child despite early treatment and apparent viral clearance. *Lancet* 2014; **384**: 1320.
93. Miller CJ, Li Q, Abel K *et al.* Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J Virol* 2005; **79**: 9217–9227.
94. Zhang ZQ, Wietgrefe SW, Li Q *et al.* Roles of substrate availability and infection of resting and activated CD4+ T cells in transmission and acute simian immunodeficiency virus infection. *Proc Natl Acad Sci U S A* 2004; **101**: 5640–5645.
95. Strain MC, Little SJ, Daar ES *et al.* Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J Infect Dis* 2005; **191**: 1410–1418.
96. Ananworanich J, Schuetz A, Vandergeeten C *et al.* Impact of multi-targeted antiretroviral treatment on gut T cell depletion and HIV reservoir seeding during acute HIV infection. *PLoS One* 2012; **7**: e33948.
97. Archin NM, Vaidya NK, Kuruc JD *et al.* Immediate antiviral therapy appears to restrict resting CD4+ cell HIV-1 infection without accelerating the decay of latent infection. *Proc Natl Acad Sci U S A* 2012; **109**: 9523–9528.
98. Hocqueloux L, Avettand-Fenoel V, Jacquot S *et al.* Long-term antiretroviral therapy initiated during primary HIV-1 infection is key to achieving both low HIV reservoirs and normal T cell counts. *J Antimicrob Chemother* 2013; **68**: 1169–1178.
99. Jain V, Hartogensis W, Bacchetti P *et al.* Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. *J Infect Dis* 2013; **208**: 1202–1211.
100. Laanani M, Ghosn J, Essat A *et al.* Impact of the timing of initiation of antiretroviral therapy during primary HIV-1 infection on the decay of cell-associated HIV-DNA. *Clin Infect Dis* 2015; **60**: 1715–1721.
101. Ananworanich J, Chomont N, Eller LA *et al.* HIV DNA set point is rapidly established in acute HIV infection and dramatically reduced by early ART. *EBioMed* 2016; **11**: 68–72.
102. Koelsch KK, Boesecke C, McBride K *et al.* Impact of treatment with raltegravir during primary or chronic HIV infection on RNA decay characteristics and the HIV viral reservoir. *AIDS* 2011; **25**: 2069–2078.
103. Ananworanich J, Dube K, Chomont N. How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs? *Curr Opin HIV AIDS* 2015; **10**: 18–28.
104. Rouzioux C, Hubert JB, Burgard M *et al.* Early levels of HIV-1 DNA in peripheral blood mononuclear cells are predictive of disease progression independently of HIV-1 RNA levels and CD4+ T cell counts. *J Infect Dis* 2005; **192**: 46–55.
105. Williams JP, Hurst J, Stohr W *et al.* HIV-1 DNA predicts disease progression and post-treatment virological control. *Elife* 2014; **3**: e03821.
106. Li JZ, Etemad B, Ahmed H *et al.* The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption. *AIDS* 2016; **30**: 343–353.
107. Chun TW, Justement JS, Murray D *et al.* Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication. *AIDS* 2010; **24**: 2803–2808.
108. Frange P, Faye A, Avettand-Fenoel V *et al.* HIV-1 virological remission lasting more than 12 years after interruption of early antiretroviral therapy in a perinatally infected teenager enrolled in the French ANRS EPF-CO10 paediatric cohort: a case report. *Lancet HIV* 2016; **3**: e49–e54.
109. Violari A, Cotton M, Kuhn L *et al.* Viral and host characteristics of a child with perinatal HIV-1 following a prolonged period after ART cessation in the CHER trial. *IAS Conference*. 2017. Paris, France. Abstract TUPDB0106LB.
110. Ananworanich J, Robb ML. The transient HIV remission in the Mississippi baby: why is this good news? *J Int AIDS Soc* 2014; **17**: 19859.
111. Chun TW, Moir S, Fauci AS. HIV reservoirs as obstacles and opportunities for an HIV cure. *Nat Immunol* 2015; **16**: 584–589.
112. Fiebig EW, Wright DJ, Rawal BD *et al.* Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS* 2003; **17**: 1871–1879.
113. Stramer SL, Glynn SA, Kleinman SH *et al.* Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. *N Engl J Med* 2004; **351**: 760–768.
114. Robb ML, Eller LA, Kibuuka H *et al.* Prospective study of acute HIV-1 infection in adults in East Africa and Thailand. *N Engl J Med* 2016; **374**: 2120–2130.
115. Massanella M, Richman DD. Measuring the latent reservoir in vivo. *J Clin Invest* 2016; **126**: 464–472.
116. Ananworanich J, Mellors JW. How much HIV isalive? The challenge of measuring replication competent HIV for HIV cure research. *EBioMed* 2015; **2**: 788–789.
117. Procopio FA, Fromentin R, Kulpa DA *et al.* A novel assay to measure the magnitude of the inducible viral reservoir in HIV-infected individuals. *EBioMed* 2015; **2**: 874–883.
118. Sanyal A, Mailliard RB, Rinaldo CR *et al.* Novel assay reveals a large, inducible, replication-competent HIV-1 reservoir in resting CD4(+) T cells. *Nat Med* 2017; **23**: 885–889.
119. Bruner KM, Murray AJ, Pollack RA *et al.* Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat Med* 2016; **22**: 1043–1049.
120. Bruner KM, Pollack RA, Murray AJ *et al.* Rapid accumulation of defective proviruses complicates HIV-1 reservoir measurements. *Conference on Retroviruses and Opportunistic Infections*. 2016. Boston, MA, USA. Abstract 83.
121. Henrich TJ, Hatano H, Bacon O *et al.* HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: An observational study. *PLoS Med* 2017; **14**: e1002417.
122. Barouch DH and Deeks SG. Immunologic strategies for HIV-1 remission and eradication. *Science* 2014; **345**: 169–174.