On the Road to a HIV Cure
Moving Beyond Berlin and London

Nikolaus Jilg, MD, PhD\textsuperscript{a,b,1}, Jonathan Z. Li, MD, MMSc\textsuperscript{b,c,*}

INTRODUCTION
AIDS was first described as a new immunodeficiency syndrome in 1981.\textsuperscript{1} The relatively short history of human immunodeficiency virus (HIV) medicine is marked by major successes and breakthroughs in research, leading to the development of specific therapies that turned a once uniformly deadly disease into a chronic carrier state and facilitates lives not affected by complications of HIV/AIDS for most people on antiretroviral therapy (ART).\textsuperscript{2} Despite this impressive advance, there is still no strategy for HIV remission or cure that can be readily employed in clinical practice.

KEYWORDS
- HIV • HIV persistence • HIV cure • HIV vaccine
- Broadly neutralizing antibodies (bNAb) • Long-term remission
- Posttreatment controllers • PTC

KEY POINTS
- A reliable therapeutic approach to human immunodeficiency virus (HIV) cure is currently not available for clinical practice.
- Cure is either defined as a complete elimination of any replication-competent virus, sterilizing cure, or a functional cure characterized by long-term remission despite remaining replication-competent virus.
- The latent reservoir consisting of silent proviruses integrated into cellular DNA is the main obstacle to cure.
- Numerous strategies for the depletion of the latent reservoir have been investigated with some promising results.
- Examples of people with long-term nonprogression and HIV remission, like the case of the Berlin patient, have amplified optimism about the general feasibility of a cure.

INTRODUCTION
AIDS was first described as a new immunodeficiency syndrome in 1981.\textsuperscript{1} The relatively short history of human immunodeficiency virus (HIV) medicine is marked by major successes and breakthroughs in research, leading to the development of specific therapies that turned a once uniformly deadly disease into a chronic carrier state and facilitates lives not affected by complications of HIV/AIDS for most people on antiretroviral therapy (ART).\textsuperscript{2} Despite this impressive advance, there is still no strategy for HIV remission or cure that can be readily employed in clinical practice.

Disclosures: Dr J.Z. Li has consulted for Quest Diagnostics and Jan Biotech.
\textsuperscript{a} Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA; \textsuperscript{b} Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA; \textsuperscript{c} Brigham and Women’s Hospital, 75 Francis Street, Boston, MA 02115, USA
\textsuperscript{1} Present address: 65 Landsdowne Street, Room 421, Cambridge, MA 02139.
\textsuperscript{*} Corresponding author. 65 Landsdowne Street, Room 421, Cambridge, MA 02139.
\textbf{E-mail address:} jli@bwh.harvard.edu

https://doi.org/10.1016/j.idc.2019.04.007
0891-5520/19/© 2019 Elsevier Inc. All rights reserved.
The authors discuss the relevance of HIV persistence as the major obstacle to cure. Next, they present several intriguing cases that have informed the field and have entertained hopes of long-term remission and cure, including the case of 1 single person, who is by many seen as the only example to date of a human being cured of HIV. Different methods are being studied or proposed as candidate therapies for cure and are described here.

**CONTENT**

**Why Does HIV Persist?**

Following an initial rapid decline in HIV-DNA in the first year on ART, the decay of the HIV reservoir slows down and eventually plateaus beyond year 4 of therapy. Cessation of suppressive therapy at any time leads to viral rebound, typically seen within 2 to 4 weeks for most individuals. What are the mechanisms behind HIV persistence?

**Active viral replication**

The presence of viral evolution in lymphoid tissue has been reported in participants on ART with undetectable viremia, leading to the proposal of active viral replication as a contributing factor to viral persistence. This cryptic viremia might explain the abnormal immune activation and inflammation seen in people on ART. Data from some treatment intensification studies provided support for this hypothesis, where the addition of an integrase inhibitor in individuals already on suppressive ART led to an increase of short-lived episomal HIV-DNA (2-LTR circles), reflecting new rounds of viral infection. There has been controversy, as the article by Lorenzo-Redondo and colleagues involved relatively few individuals, all with recent ART initiation, and concerns have been raised about the methodology. In contrast, there was no evidence of viral replication or evolution on ART in several other studies, including those with ART intensification. Viral replication on ART was detected in tissue compartments, such as the B cell follicles in lymph nodes, in non-human primate (NHP) elite controllers, which was explained by the absence of antiviral CD8+ cells in the B cell follicles. Similarly, replication competent cells were found in lymph nodes of aviremic patients. Low drug concentrations in B cell follicles may be a contributing factor. HIV sanctuary sites in certain tissues may be a relevant part of the reservoir, but these are often not easily accessible for studies. There was no viral replication detected on longitudinal tissue sampling of ART-suppressed individuals in 1 study. Finally, suboptimal ART adherence may result in persistent low-level viral replication, even for individuals with apparent viral suppression by commercial viral load assays.

**Prolonged cellular survival**

HIV integrates into the host genome and persists as provirus during the lifetime of the cell. Most cells die shortly after infection, either due to cytopathic effects of the virus or host immune responses, but in latency, HIV is transcriptionally/translationally silent and allows these cells to evade the immune response. Identification of the small fraction of HIV-infected CD4+ cells, that form the reservoir and cause rebound when ART is stopped, has been a major focus of HIV cure research. T memory stem cells are a particularly long-lived subset of central memory T cells carrying proviral DNA in relatively high frequency. The latent reservoir consists mainly of intact proviruses in quiescent cells and might have been considerably underestimated in many studies. Hematopoietic stem and progenitor cells (HSPCs) that are capable of lifelong survival, self-renewal, and clonal expansion may be infected and contribute to the viral reservoir.
Homeostatic or clonal proliferation

Homeostatic proliferation, the physiologic response to maintain T cell numbers, and clonal proliferation of infected cells are increasingly recognized as key mechanisms behind the maintenance and expansion of the HIV reservoir. HIV preferably integrates into regions of actively transcribed genes, particularly genes that are involved in oncogenesis or cell-cycle control. A large fraction of the cells that carry proviruses was shown to be of clonal origin. While most of these only harbor defective provirus, some clonally expanded CD4$^+$ T cells do indeed produce infective virus in vivo. One report suggested that more than 99% of infected cells belong to clonal populations after a year of ART.

Has There Been a Cure? Notable Examples and Strategies Toward Control and Elimination of Human Immunodeficiency Virus

An HIV “cure” may either refer to sterilizing cure, meaning that there is no remaining virus capable of reactivation left in the body, versus functional cure, a state of long-term remission in that intact virus or proviral sequences are still present, but are being controlled and the disease does not progress. There have indeed been several well-described examples of patients who have achieved long-term remissions, some where prematurely considered cured by the non-scientific community.

Why should “cure” matter in the times of highly successful and well-tolerated ART? Some people are not well controlled by or do not tolerate currently available regimens due to factors such as drug resistance, limited access to ART and medical care in many areas of the world, comorbidities (eg, cardiovascular and metabolic diseases), pill fatigue, and the stigma still associated with HIV infection, that are persisting challenges with current ART. Finally, life-long ART accrues a significant cumulative cost.

Stem Cell Transplantation

The Berlin and London patients

A 2009 case report described long-term remission of HIV infection reviving optimism regarding feasibility of a cure: the so-called Berlin patient had been HIV positive and on suppressive ART when he developed acute leukemia (unrelated to HIV) requiring a myeloablative allogeneic hematopoietic stem cell transplant (HSCT). After HSCT from a matching donor who was additionally selected for homozygosity of CCR5 Δ32, a 32-base-pair deletion in the CCR5 gene that renders the host cells resistant to viral entry, the Berlin patient has remained off combined ART without evidence of residual virus so far (>10 years) despite an extensive search. Hence, most experts consider him the first documented example of a sterilizing HIV cure, although negative tests cannot completely exclude the presence of intact virus everywhere in the patient’s body. In a recently published report, there is hope that a second patient may now be free of HIV. This individual, known as “the London patient”, received an allo-HSCT for Hodgkin’s lymphoma from a donor homozygous for the CCR5Δ32 mutation. This patient has been in HIV remission for 18 months after ART discontinuation with undetectable plasma viral load by an ultrasensitive assay and undetectable HIV DNA in peripheral CD4 T cells. As the authors point out, the duration off ART for this individual is far shorter than that of the Berlin patient and it is premature to conclude that this patient has been cured. In contrast to the Berlin patient, the London patient received only one transplant that included a reduced intensity conditioning regimen without whole body irradiation, suggesting that a less intensive approach may be sufficient to induce HIV remission.
The Boston patients and other examples of allogeneic hematopoietic stem cell transplantation in people with HIV infection

In addition to administration of donor cells homozygous for the CCR5 Δ32 mutation, the Berlin patient’s treatment also included chemotherapy, whole-body irradiation, and a second transplantation from the same donor after a relapse of his leukemia. Two men with HIV infection and hematologic malignancies in Boston received allogeneic HSCT from donors who did not have the CCR5 Δ32 mutation, leaving the donor T cells susceptible to HIV infection. While there was a significant reduction of the viral reservoirs, both patients eventually rebounded, albeit with a significant delay of 12 and 32 weeks after cessation of ART.32,33 Another patient who underwent allogeneic HSCT with donor cells harboring wild-type CCR5 experienced rebound after more than 9 months.34 These results point to a pivotal role of the CCR5 Δ32 allele in preventing the resurgence of infection, although the rarity of the homozygous genotype in the donor pool and the toxicity of allo-HSCT have made it challenging to replicate the Berlin patient’s experience.35–37 The ICiStem consortium (International Collaboration to guide and investigate the potential for HIV cure by Stem Cell Transplantation) found a profound reduction in the HIV reservoirs after allogeneic HSCT in 6 individuals.38 Finally, autologous stem cell transplantation or cytoreductive chemotherapy in the absence of HSCT led only to a transient reduction of the reservoir followed by recovery and even expansion of reservoir size.39

Allogeneic hematopoietic stem cell transplantation as a strategy

Current allogeneic bone marrow transplantation is fraught with high morbidity and mortality prohibiting its use for HIV infection other than in patients with an accepted medical indication for HSCT. Further attempts with hematopoietic stem cells (HSCs) from CCR5 Δ32 homozygous donors led to reduction of the reservoir and transient viral control, but were limited by high mortality.35,40,41 Of note, routine screening for CCR5 Δ32 has recently been established in several cord blood and bone marrow banks and facilitates identification of HLA identical donors that are also homozygous for CCR5 Δ32, increasing the chance for a respective match to 20%-25% for patients with Central European ancestry.42 One patient experienced viral escape after HSCT from a CCR5 Δ32 positive donor which was caused by the emergence of CXCR4 tropic virus, while pre-interventional HIV testing had shown CCR5 tropism.40,41 Moreover, CCR5 Δ32 homozygous individuals with HIV have been identified, illustrating once more that the mutation does not confer complete resistance to infection.43

Gene Therapy

Host gene modification

Molecular approaches to altering host factors, offer the prospect of reproducing the Berlin patient’s experience while avoiding the morbidity of allogeneic HSCTs. Zinc-finger nucleases (ZNF) have been used to knockout CCR5 in both CD4+ T cells and CD34+ hematopoietic stem and progenitor cells (HSPCs). CCR5 editing in autologous cells of HIV+ subjects was safe in a phase 1 trial, and several subsequent early phase trials have used the technique.44,45 More recently developed gene editing tools like TALEN and CRISPR-Cas9 will probably be preferred going forward because of their relative ease of use and improved specificity. These techniques still bear the risk of off-target effects leading to insertions, deletions, and point mutations, including some that may only manifest after long periods of time, which currently prohibits their routine use in humans.46 Knocking out both coreceptors may provide broad protection from viral entry. While a specific, systemic CCR5 knockdown may be well tolerated as suggested by the naturally occurring CCR5 Δ32 mutation, CXCR4 has...
important functions in bone-marrow mobilization, and systemic knockdown may not be safe. There are, however, promising results by Didigu and colleagues,\textsuperscript{47} who used ZFNs to knock out both coreceptors in CD4\textsuperscript{+} T cells ex vivo and infused them into humanized HIV+ mice, which did not cause any apparent functional immune defects. T cell proliferation is expected to amplify effects of genetically altered T cells because these HIV-resistant populations are expected to replace HIV-susceptible host T cells that are depleted in the setting of active infection.

**Proviral inactivation**

Besides their use for targeting host factors, ZNFs, TALENs, and CRISPR-Cas9 have been used to directly disrupt proviral DNA: an HIV-specific CRISPR-Cas9 with a lentiviral vector suppressed viral replication in humanized mice after engraftment of patient-derived peripheral blood mononuclear cells (PBMCs).\textsuperscript{48} Based on the Cre/loxP system, Karpinski and colleagues\textsuperscript{49} have created a recombinase (Brec1), which site specifically recognizes a highly conserved 34-bp sequence in the long terminal repeats (LTRs) allowing for precise excision of proviral DNA. The strategy led to elimination of provirus from patient-derived infected cells in ex vivo experiments and achieved HIV eradication in a humanized mouse model. Off-target effects were not detected. Efficient, reliable, and safe delivery of the relevant molecules to humans has been the hurdle to using these molecular technologies in clinical studies.

**Early Therapy for Cure and Posttreatment Control**

**The Mississippi baby and the role of very early treatment**

A girl who had perinatally contracted HIV and was started on ART 30 hours after birth, then had stopped ART after 18 months when she was lost to follow-up. She found to be aviremic when reestablishing care after 12 months off therapy.\textsuperscript{50} This case nurtured hopes that very early treatment initiation might prevent establishment of latency. The child eventually relapsed after 27 months off ART.\textsuperscript{51} While disappointing, the prolonged period of HIV remission in this case further supported the hypothesis that early treatment restricts the seeding of the viral reservoir and may increase the chances of sustained HIV remission, at least in infants. Other treatment interruption studies of adults who had initiated ART during the very early stages of infection (Fiebig I and Fiebig II, ie, before specific HIV-antibodies are detectable) failed to demonstrate that early treatment prevented the establishment of HIV infection.\textsuperscript{52,53} As discussed later, early initiation of ART may, however, increase the chances of sustained HIV remission.\textsuperscript{54–56}

**The VISCONTI cohort and early capture studies**

ART-free remission, that is, functional cure, following early treatment initiation, like in the case of the Mississippi baby, was also studied in the VISCONTI cohort (Viro-Immunological Control after Treatment Interruption). Sáez-Cirión and colleagues\textsuperscript{55} identified 14 patients from a large database that began ART during primary infection, who controlled viremia and preserved CD4\textsuperscript{+} T cell counts for several years after ART interruption. Of note, these individuals started therapy within 2 months of infection, but considerably later than in the examples above, that is, during Fiebig stage III to V for almost all participants, when HIV-specific antibodies are already reliably detectable. Individuals that display HIV control after, but not before ART, like the participants of the VISCONTI cohort, were termed posttreatment controllers (PTCs).

Early capture cohorts aim to systematically and prospectively study the effects and outcomes of early treatment initiation by continuously screening at-risk populations in highly endemic areas (eg, South Africa, East Africa, Thailand) and start treatment as soon as participants turn positive, which may further shed light on PTC frequency amongst patients treated within a narrow, early timeframe.\textsuperscript{57,58}
Further posttreatment controllers studies
A central question is how ART facilitates viral control by the immune system after treatment cessation. Several observations support a lower reservoir size during therapy in PTCs versus noncontrollers. Namazi and colleagues have recently identified and characterized 67 PTCs from existing trials. There was a significantly higher probability of achieving PTC status in the participants with treatment starting during early versus chronic infection (13% vs 4%; P<.001).

Latency Modifying Agents
“Shock and kill” or “kick and kill” has been proposed as a strategy well suited to overcome the specific obstacles of latency. It involves the seemingly at first counterintuitive activation of the reservoir which results in viremia and is important for 2 reasons: first, latently infected cells are, per definition, not making any viral molecules and therefore remain invisible to the immune system; second, non-replicating proviruses do not provide any targets for specific antiviral drugs. Hence, forcing these long-lived cells out of latency is a way to uncover them. Once activated, mean survival of infected CD4+ T cells would hypothetically be quite short, due to both the cytopathic effect of the virus and the unfolding immune response, but there is evidence that the immune system actually has trouble clearing these cells efficiently. The “kick” by latency reversing agents (LRAs) is followed by interventions that promote killing of infected cells, typically while measures like ART would protect from new rounds of infection. Purging the body from latently infected cells would diminish or eliminate the reservoir. A number of drugs that are already in clinical use were identified as LRAs, which expedites human trials in HIV infection. These drugs include valproic acid, disulfiram, histone deacetylase inhibitors (HDACi), protein kinase C agonists, and Toll-like receptor (TLR) agonists. Despite significant increases of viral transcription and replication in multiple trials, there has been no relevant reduction in the viral reservoir dampening the initial excitement about the strategy. A potential explanation for this finding is that many LRAs may not reach high enough levels to activate the cells in the relevant compartments or may only be capable of activating a small fraction of proviruses. Another concern regarding these agents are off-target effects, such as activation of uninfected T cells, which may render them susceptible to HIV, and unwanted activation of additional cell types that may, for example, cause reactivation of other latent viruses, like the herpes family viruses or human T cell lymphotropic virus (HTLV). “Block and lock” or “soothe and snooze” is an approach that would eventually cause a state of deep viral hibernation by means of latency securing agents, ideally lowering the risk of proviral reactivation to 0. Didehydrocortistatin A, a specific inhibitor of the HIV protein Tat (transactivator of transcription), has been studied for this purpose. Tat is crucial for HIV reactivation and is therefore an attractive target for proviral silencing strategies. Short hairpin RNA (shRNA) targeting the HIV promoter region in the long terminal repeats (LTRs) was cloned into lentiviral vectors and protected against reactivation by LRAs in cell culture models. The proteasome is a key contributor to viral latency, which at least partially is achieved by breaking Tat down, and thereby blocking viral transcription. Thus, proteasome inhibition reverses latency, whereas inactivating Tat promotes it.

Antibody Therapy
In virology, no antibody (Ab) responses have been as extensively studied as those to HIV. Ab-based therapies have been explored for HIV prevention and control, and applications aiming for cure may involve a delivery system of Ab genes to humans facilitating sustained in vivo Ab production. Broadly neutralizing antibodies (bNAb)
target highly conserved regions of the envelope protein (Env) occur naturally in a minority of HIV-infected individuals. A limited number of highly selected candidate monoclonal antibodies have been studied: in a humanized mouse model, therapy with a specific bNAb not only led to viral neutralization but also led to enhanced clearance of HIV-infected cells. Several groups have tested bNAbs in the simian-human immunodeficiency virus (SHIV) infection model in non-human primates (NHP) and have demonstrated that specific bNAbs protect from new infection, reduce plasma viral loads, extend time to viral rebound, and reduce PBMC and lymph node proviral DNA levels. Treatment with a single monoclonal Ab appears prone to resistance, but combination therapy likely obviates resistance mutations. In a 2018 proof-of-principle paper, a bioengineered trispecific Ab combining specificities to 3 independent HIV envelope determinants conferred complete immunity against SHIV infection in monkeys. Several monoclonal bNAbs have been shown to reduce viremia in HIV-positive, untreated individuals, and delay time to rebound in ART-treated subjects after analytical treatment interruption. Widespread tissue penetration may be one of the advantages of Ab therapy, because ART may not reach adequate drug levels in all relevant compartments. Borducchi and colleagues have recently outlined a promising strategy to deplete the viral reservoir by using a combination of ART, a TLR7 agonist (vesatolimod), and a bNAb (PGT121) in SHIV-infected monkeys: TLR7 acts as an antiviral through type I interferon-dependent activation of immune cells, but moreover, it has LRA properties, potentially due to the induction of CD4+ T cell activation. NHPs on ART that had been treated with both vesatolimod and PGT121 experienced diminished viral rebound kinetics after treatment cessation compared with the sham or monotherapy groups.

**Therapeutic Vaccination**

Preventative HIV vaccine trials have generally been disappointing, and only 1 clinical study has demonstrated a modest protective effect from new infection (31%) when given to HIV-negative individuals. After some of these study subjects acquired HIV, but there was no noticeable effect on degree of viremia or CD4+ T cell numbers induced by the vaccine. As noted above, there is evidence that reversal of HIV latency may not necessarily lead to clearance of reactivated cells, and a number of therapeutic vaccine strategies have been tested. While the overall track record of therapeutic vaccines has been disappointing, there may be a modest effect of immunization on viral set point. In view of the encouraging results from work with bNAbs (discussed above), a vaccine that could trigger production of highly effective bNAbs would theoretically be a powerful therapeutic. It remains unclear to which degree neutralizing, or nonneutralizing antibodies, T cell, NK cell, or other specific immune responses are necessary for an adequate immune response to HIV.

**SUMMARY**

Two well documented cases of HIV long-term remission - and possible cure - have raised the hope that curative treatments may one day be available in clinical practice to replace ART. There are many promising leads for targeting the viral reservoir, including therapeutic latency reversal, molecular host factor modification, silencing or excision of the provirus, administration of monoclonal broadly neutralizing antibodies, and therapeutic immunizations. It is likely that a combination of strategies will be required for cure. The Berlin and London patients remain a living testament to the feasibility of HIV remission in the absence of ART. The remaining challenge is...
how to develop reliable and safe curative therapies to make them available to people living with HIV.

ACKNOWLEDGMENT

Dr. Jilg was supported by the National Institutes of Health Multidisciplinary AIDS Training grant (AI007387, PI: Dr. D. Kuritzkes). Dr. Li was supported by the Harvard University Center for AIDS Research (CFAR) (National Institute of Allergy and Infectious Diseases grant number 5P30AI060354-08) and a subcontract from UM1 AI106701 to the Harvard Virology Specialty Laboratory.

REFERENCES


34. Cummins NW, Rizza S, Litzow MR, et al. Extensive virologic and immunologic characterization in an HIV-infected individual following allogeneic stem cell...


42. Hutter G. The cure of Timothy Brown. How is his condition now and has this case been repeated? MMW Fortschr Med 2018;160(Suppl 2):27–30 [in German].


