Novel Approaches To Curing HIV

HIROYU HATANO, MD, MHS
Assistant Professor of Medicine
University of California at San Francisco
San Francisco, California

One of the greatest advances in modern medicine has been the development of highly active antiretroviral therapy (HAART) for the treatment of HIV. HAART decreases HIV-associated morbidity and mortality for patients with regular access to these medications, but it does not appear to completely restore their health for reasons that currently are unclear. Patients doing well on effective HAART remain at risk for cardiovascular, renal, and liver disease, as well as cancer, osteopenia, and other non-AIDS-defining events. Additionally, HAART requires daily adherence to medicines that all have some inherent potential for side effects. Finally, despite several important recent advances in HIV prevention methods, the epidemic continues to grow both locally and globally. As a result, there has been an increased interest in ways to fully eradicate HIV from infected individuals (ie, a "sterilizing cure"), or to at least achieve a “functional cure.”

Cure Models for HIV

A Sterilizing Cure Model: The ‘Berlin Patient’

HIV infection of CD4-positive T cells requires the presence of the CD4 T-cell receptor, along with one of the chemokine receptors, C-C chemokine receptor 5 (CCR5) or, less commonly, C-X-C chemokine receptor 4 (CXCR4). Homozygosity for a 32-base pair deletion in the CCR5 gene (CCR5-Δ32) has been known to significantly decrease susceptibility to HIV infection. The “Berlin patient” is an HIV-infected individual who, despite effective treatment with HAART, developed acute myelogenous leukemia and subsequently received 2 allogeneic stem cell transplants from an HLA-identical donor who had been screened for homozygosity for the CCR5-Δ32 mutation. Although there has been some controversy as to whether a sterilizing cure has been achieved in this patient, 5 years have passed since the initial stem cell transplant and based on both published and emerging data, it appears that the patient has at least been clinically cured of HIV. He has maintained undetectable levels of plasma HIV RNA using conventional assays and has remained disease-free despite discontinuing HAART 1 day after his initial stem cell transplant occurred 5 years ago. However, it remains to be determined which, if not
all of the treatments that the patient received—including multiple cycles of chemotherapy, antithymocyte globulin, and total body irradiation—was responsible for his cure. The “Berlin patient” represents a single occurrence of a cure that resulted from a series of treatments, and this therapeutic course would be difficult to justify in the majority of patients. But this patient and his willingness to share this story clearly have galvanized both the HIV-infected community and those involved in HIV cure research.

A Functional Cure Model: HIV-infected Controllers

Although complete eradication may not be possible for most patients infected with HIV, a “functional cure,” a state in which patients are able to maintain undetectable viral loads without therapy, may be possible. The best evidence for a functional cure is the so-called “elite” controllers, who are defined as HIV-seropositive individuals with plasma HIV RNA levels below the level of conventional detection without HAART. Elite controllers may be conceptualized as a rare but naturally occurring model of a functional HIV cure (or “HIV remission”), and they are ideal patients in which to study both HIV persistence and the possibility of eradication.

Studies have shown that:
1. The vast majority of elite controllers have low but detectable plasma HIV RNA, cell-associated RNA in peripheral blood mononuclear cells (PBMCs), and proviral DNA in PBMCs if ultrasensitive assays are used.15-17
2. Elite controllers have readily measurable levels of HIV RNA and DNA in gut-associated lymphoid tissue (GALT),18 which is known to be a key reservoir site in HAART-suppressed noncontrollers.19-22
3. A significant proportion of elite controllers are infected with replication-competent virus23,24 and not with virus that contains significant genetic defects.25
4. Elite controllers have higher levels of immune activation compared with HAART-suppressed and HIV-negative individuals.26
5. Elite controllers have higher levels of atherosclerosis (as measured by intima-media thickness) compared with uninfected individuals, even after adjusting for traditional risk factors for cardiovascular disease.27
6. Some elite controllers with high levels of immune activation immunologically progress to AIDS despite maintaining virologic control without HAART.26 Collectively, these data suggest that despite the unique ability of these individuals to control plasma

Table. Select Research Toward a Cure for HIV

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Purpose</th>
<th>Design</th>
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<tbody>
<tr>
<td>NCT01025427</td>
<td>To characterize the viral reservoir in blood and lymphoid tissues of HIV-infected controllers and to study the effects of HAART on viral dynamics and host immune responses</td>
<td>Prospective, nonrandomized, open-label study of raltegravir/tenofovir/emtricitabine for 24 wk</td>
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<tr>
<td>ACTG 5308</td>
<td>To understand the effects of ART on immune activation and HIV reservoirs in HIV-infected controllers</td>
<td>Prospective, nonrandomized, open-label study of fixed-dose rilpivirine/emtricitabine/tenofovir for 48 wk</td>
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<tr>
<td>NCT01365065</td>
<td>To assess the safety and ability of vorinostat to activate dormant HIV-infected CD4 T cells, as measured by unspliced RNA</td>
<td>Open-label study of vorinostat 400 mg PO qd for 14 d</td>
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<tr>
<td>ACTG 5301</td>
<td>To assess the safety, pharmacokinetics, and ability of an anti-PD-1 antibody to decrease the size of the latent HIV reservoir</td>
<td>Open-label, single-dose study of MK3475, an anti-PD-1 antibody</td>
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<tr>
<td>NCT01535235</td>
<td>To determine whether the use of an ACE inhibitor in addition to HAART reverses lymphoid fibrosis, improves HIV-specific host immune responses, and accelerates clearance of the latent reservoir</td>
<td>Randomized, double-blind, placebo-controlled trial of lisinopril 20 mg qd for 24 wk</td>
</tr>
<tr>
<td>ACTG 5317</td>
<td>To investigate the effect of telmisartan on fibrotic and inflammatory contributions to end-stage organ disease in patients on ART with well-controlled HIV</td>
<td>In development</td>
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ACE, angiotensin-converting enzyme; ACTG, AIDS Clinical Trials Group; ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; NCT, National Clinical Trial; PD-1, programmed cell death protein-1; PO, orally; qd, once daily

Based on information from ClinicalTrials.gov and ACTGnetwork.org.
viremia to very low levels, viral control is occurring at a “cost” to their immune systems, which may lead to a higher risk for AIDS- and non-AIDS-defining events than that observed in uninfected adults. Our research group has recently completed a pilot study in which a small cohort of HIV-infected controllers were prospectively treated with HAART to characterize the viral reservoir in blood and lymphoid tissues in controllers, as well as to study the effects of HAART on viral dynamics and host immune responses (ClinicalTrials.gov NCT01025427; see Table). A similar, larger study currently is being developed through the AIDS Clinical Trials Group (ACTG 5308). Ultimately, these studies may provide novel insights into which immunologic and virologic factors are (and are not) necessary for a functional cure so that this knowledge can be applied to advancing efforts toward a cure for HIV noncontrollers.

**Multipronged Approaches to a Cure**

A successful cure strategy for the majority of patients with HIV will likely require a combination approach that includes early access to antiretroviral drugs, anti-retroviral drugs or other adjunctive therapies that provide effective penetration of antiretroviral drugs into tissue reservoirs, agents to “purge” the viral reservoir, interventions to boost host immune responses against HIV, and therapies that make uninfected host cells resistant to infection. Novel interventions that are currently being investigated as components of a successful approach toward a cure are discussed below. If any of these interventions provide positive results as single agents, the ultimate goal would be to test them in combination with other viable interventions.

**Interventions To Purge the Viral Reservoir**

There is still considerable controversy as to whether low-level viral replication persists in the setting of suppressive HAART. Several recent intensification studies have addressed this issue. Although the studies differed in terms of patient populations and outcome measures, they consistently found that intensification does not appear to decrease plasma viremia, as measured by ultrasensitive plasma HIV RNA assays. However, one study reported a significant decrease in viral replication following intensification, as measured by an increase in 2-long terminal repeat circles in PBMCs (a marker of recent viral replication). Another study reported a decrease in unspliced HIV RNA in GALT. These latter studies suggest that intensification may decrease viral replication if measured in cells and tissues.

Despite the complete or nearly complete inhibition of viral replication with HAART, replication-competent HIV persists indefinitely in all individuals and HIV is able to be detected in PBMCs and lymphoid tissues in long-term HAART-suppressed individuals. This may be due to the inability of current antiretroviral drugs to inhibit the cell-to-cell transfer of HIV, although this remains a controversial topic. Most importantly, HAART cannot cure HIV infection on its own due to the persistence of the virus in a small pool of latently infected and long-lived CD4-positive T cells. Several agents have been suggested to purge these reservoir cells, such as vorinostat, a histone deacetylase inhibitor that increases DNA transcription and is approved for the treatment of cancer. A recently published, small open-label study in the United States showed that a single dose of vorinostat increased the expression of cell-associated RNA in a subset of individuals on long-term HAART. A larger study currently is underway in Australia that will evaluate the virologic effects of administering 2 weeks of vorinostat in HAART-suppressed individuals (ClinicalTrials.gov NCT01365065).

An alternate approach is to identify and reverse host mechanisms that result in the establishment of viral latency. One intervention that is currently being developed is an anti–programmed cell death protein 1 (PD-1) antibody. PD-1 is a member of the CD28/CTLA-4 family of T-cell regulators that negatively regulates the inflammatory response. Due to the potential harm that can occur during an inflammatory response, immune activation is associated with immediate and often potent immunoregulatory responses. These immunoregulatory responses include the upregulation of pathways that reverse a cell’s activated state (ie, “negative regulators”). These less-activated cells likely persist for prolonged periods of time and may contribute to HIV persistence. PD-1 expression is upregulated on activated CD4- and CD8-positive T cells, along with HIV-specific CD8-positive T cells. Additionally, PD-1-expressing CD4-positive T cells have been shown to be a preferential reservoir for HIV. Finally, PD-1 expression on HIV-specific T cells has been associated with T-cell exhaustion and disease progression. Thus, it is hypothesized that the administration of an anti–PD-1 antibody will decrease the size of the latent reservoir by increasing HIV transcription and by enhancing the effector functions of CD8-positive T cells. An open-label, single-dose protocol currently is being developed (ACTG 5301).

**Interventions To Improve Host Immune Responses**

In a recent in vitro study, however, the reversal of HIV latency itself did not lead to cell death, and the addition of potent cytotoxic T lymphocytes was necessary to kill latently infected cells. Our group has shown that in treated individuals who have complete or nearly complete suppression of viral replication, those with stronger HIV-specific mucosal responses in GALT have lower measures of latent viral reservoirs. This is consistent with studies of elite controllers in which mucosal T-cell responses have been associated with the control of HIV replication. These data suggest that approaches aimed at expanding HIV-specific responses in gut mucosa and other lymphoid tissues, where the majority of the viral reservoir resides, may accelerate clearance of the reservoir and may be an important part of a multipronged approach to HIV eradication.

In addition to the continued development of therapeutic HIV vaccines, other interventions that boost
the host immune response currently are being investigated. One approach is the use of antifibrotic agents to decrease lymphoid fibrosis, improve HIV-specific responses, and decrease the size of the viral reservoir. It has been shown that collagen deposition in lymphoid tissues occurs early in HIV infection, does not normalize with HAART, limits immune reconstitution, predicts the degree of HAART-mediated immune restoration, and importantly, may limit the host immune response to HIV. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers have been shown to have antifibrotic effects in other clinical settings through the inhibition of transforming growth factor-β. Our research group (ClinicalTrials.gov NCT01535235) and several others (ACTG 5317) currently are investigating these classes of drugs to determine whether they are antifibrotic in the setting of treated HIV infection, and whether they are able to improve HIV-specific immune responses and decrease the size of the viral reservoir in lymphoid tissues.

**Making Uninfected Host Cells Resistant to Infection: Gene Therapy**

As stated above, HIV infection requires the presence of the CD4 receptor, as well as the presence of one of the chemokine receptors (eg, CCR5 or, less commonly, CXCR4). Homozygosity for the 32-base pair deletion in CCR5 significantly decreases susceptibility to HIV infection, whereas heterozygosity for the CCR5 deletion can confer improved HIV disease outcomes. This knowledge led investigators to develop an HIV-resistant genotype using zinc-finger nucleases (ZFNs) to disrupt endogenous CCR5. First, autologous CD4-positive T cells are removed from the HIV-infected individual, then endogenous CCR5 is genetically modified with ZFN to create CCR5 ZFN-modified CD4-positive T cells. The CCR5 ZFN-modified CD4-positive T cells are expanded ex vivo and then reinfused into the patient. Preliminary results suggest that the intervention is safe, well tolerated, and remains an important area for continued research.

**Future Steps for HIV Cure Research**

To sustain the current momentum in HIV cure research, we need a continued collaboration between the HIV-infected community, researchers, ethics professionals, and funding sources. Groundbreaking advances in both the treatment and prevention of HIV have been entirely dependent on the willingness of an informed HIV-infected community to engage in patient-centered interventional studies—all of which carry some inherent risks. There should be a continued collaboration between clinic-based translational researchers and laboratory-based basic scientists. Funding agencies must be nimble and should seek out high-risk, high-impact studies that investigate novel interventions. Moreover, researchers should consider a consolidation of efforts so that studies are not unnecessarily repeated, or so that data from similar studies can be combined. This undoubtedly will require some standardization of in vitro latency assays and sampling protocols, especially for intensive tissue samples, although this may prove to be challenging. An animal model of treated HIV infection has thus far been difficult to achieve, but important progress has been accomplished in this area. Animal studies should be designed as similarly to planned human studies as possible. They should be used not only to test traditional outcomes of safety, pharmacokinetics, and pharmacodynamics, but also to specify which primary end points (eg, blood vs tissue) should be used, along with the optimal timing for measuring these end points. Ultimately, interventions should be tested in well-characterized, long-term HAART-suppressed individuals.

**References**


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