Progress Toward Curing HIV Infections With Hematopoietic Stem Cell Transplantation

Stephen T. Smiley,1 Anjali Singh,1 Sarah W. Read,1 Opendra K. Sharma,1 Diana Finzi,1 Clifford Lane,2 and Jeffrey S. Rice3

1Division of AIDS, 2Clinical and Molecular Retrovirology Section, Laboratory of Immunoregulation, and 3Division of Allergy, Immunology, and Transplantation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Combination antiretroviral therapy can suppress human immunodeficiency virus (HIV) infection but cannot completely eradicate the virus. A major obstacle in the quest for a cure is the difficulty in targeting and measuring latently infected cells. To date, a single person seems to have been cured of HIV. Hematopoietic stem cell transplantation (HSCT) preceded this cancer patient’s long-term sustained HIV remission, but researchers have been unable to replicate this cure, and the mechanisms that led to HIV remission remain to be established. In February 2014, the National Institute of Allergy and Infectious Diseases sponsored a workshop that provided a venue for in-depth discussion of whether HSCT could be exploited to cure HIV in cancer patients requiring such procedures. Participants also discussed how HSCT might be applied to a broader community of HIV-infected persons in whom the risks of HSCT currently outweigh the likelihood and benefits of HIV cure.

Keywords. HIV; transplantation; latency; reservoirs.

Since the start of the AIDS epidemic, vast resources have been dedicated to the treatment and prevention of human immunodeficiency virus (HIV) infection. Today, combination antiretroviral therapy (cART) can control and suppress viremia to near-undetectable levels but is unable to completely eradicate the virus [1, 2]. Low levels of HIV persist in patients on cART, and viremia rebounds upon cessation of therapy, typically within just a few weeks. Thus, HIV-infected persons must remain on therapy indefinitely. A number of concerns with cART, including drug interactions, toxicities, costs, viral resistance, and patient adherence, drive a desire to identify a cure. A major obstacle in the quest for a cure is the difficulty in identifying, targeting, and measuring the reservoir of latently infected cells [3, 4].

The 2009 report [5] that a patient with cancer had been cured of HIV infection following hematopoietic stem cell transplantation (HSCT) was received with much interest in both the HIV and transplantation research communities. That individual remains free of detectable HIV despite cessation of cART >7 years ago. However, researchers have been unable to replicate this patient’s cure in other HSCT recipients, and the mechanisms that led to his sustained remission have yet to be established. Several non–mutually exclusive hypotheses have been proposed: the pretransplant conditioning regimen may have reduced the number of HIV-infected cells, the transplant donor’s genetics may have prevented infection of the graft, and/or graft-vs-host (GVH) responses may have eradicated the latent HIV reservoirs.

Determining the mechanisms of HIV eradication in this patient and learning how to exploit those mechanisms to cure others will require collaboration between HIV and transplantation researchers. To that end, the National Institute of Allergy and Infectious Diseases sponsored a workshop entitled “Graft Versus Host Responses in HIV Eradication” in Bethesda, Maryland, on 10–11 February 2014. The workshop provided a venue for in-depth discussion of the potential to exploit HSCT to cure HIV infection in cancer patients requiring such procedures. Participants also discussed how HSCT might be applied to a broader community of
HIV-infected persons, in whom the risks of HSCT currently outweigh the likelihood and benefit of HIV cure.

ASSOCIATIONS BETWEEN ALLOGENEIC HSCT AND HIV CURES

Several reports have described HIV-infected patients who may have eradicated HIV subsequent to receiving allogeneic HSCT to treat a malignancy [6]. The best-documented case is that of the “Berlin patient” [5]. In 2007, this HIV-infected man with acute myeloid leukemia received myeloablative therapy and an allogeneic HSCT from a donor whose cells were resistant to HIV infection due to being homozygous for CCR5-D32, a non-functional allele of the CCR5 coreceptor used by HIV to infect human cells [7]. He developed mild GVH disease (GVHD), which was readily treated, and received a second HSCT from the same donor when his leukemia recurred. Combination ART was stopped at the time of the first CCR5-D32 transplant and he has now gone without antivirals for >7 years. He sporadically tests borderline positive for HIV nucleic acids using sensitive polymerase chain reaction (PCR)-based assays but shows no evidence of productive HIV infection [8]. His HIV-specific T-cell and antibody responses have waned [8]. He is considered cured.

In light of the Berlin report, much optimism was generated by a 2013 report that HIV eradication may have been achieved in 2 Boston patients [9]. Under the cover of cART, these men received reduced-intensity conditioning regimens followed by allogeneic HSCT from CCR5 wild-type donors. They both developed GVHD and appeared to completely clear their HIV infections. Neither had detectable viral reservoirs nor HIV-specific T-cell responses in their peripheral blood. However, unlike the Berlin patient, viremia returned in both Boston patients when they subsequently discontinued cART. Their viral rebounds occurred with greatly delayed kinetics compared with typical HIV patients, and viral genome analysis suggests that their rebounds resulted from very small numbers of infected cells (Timothy Henrich, unpublished data). These observations suggest that HSCT had significantly, albeit incompletely, reduced their HIV reservoirs.

THE CHALLENGE: LATENT RESERVOIRS OF HIV

There have been reports of other unsuccessful attempts to repeat the Berlin patient’s cure [2]. These cases highlight a significant challenge: eliminating the latent HIV reservoir. They also point to limitations in peripheral blood measurements for the assessment of potential cures, and demonstrate that current assays do not have the sensitivity required to detect low-level HIV infections.

Following HIV infection, some infected CD4 T cells differentiate into long-lived, resting memory T cells that contain integrated proviruses [3, 4]. These memory CD4 cells are believed to create a latent HIV reservoir that resists cART and is invisible to the immune system. Upon their activation, these latently infected T cells can produce HIV. Because the half-life of infected memory cells has been estimated to be 44 months, it could take >70 years for patients on fully suppressive cART to eradicate these reservoirs.

Currently, the gold standard assay for measuring reservoirs of replication competent HIV is the quantitative viral outgrowth assay [3, 4]. Unfortunately, this assay is suboptimal for cure research: It is labor intensive, requires large volumes of peripheral blood or leukapheresis, and significantly underestimates the size of the replication-competent reservoir [10]. PCR-based assays of proviral HIV DNA in peripheral blood are more sensitive but cannot distinguish replication-competent and -incompetent virus. Moreover, even PCR assays failed to detect the Boston patients’ latent reservoirs. These observations highlight the need for better assays. They also suggest there may be other, yet to be identified, HIV reservoirs.

Disrupting latency (eg, by activating latent proviral genomes in T cells) might help to eradicate HIV reservoirs. Initial efforts to this end aimed to nonspecifically activate all latently infected T cells while preventing infection of other cells with cART. The hope was that the activated, HIV-producing cells would succumb to virus-induced cytotoxicity, as had been observed in vitro. Clinical trials using interleukin 2, interleukin 7, or anti-CD3 to this end highlighted a number of complexities, including that latently infected T cells persist despite reactivation in the presence of cART [2]. Current efforts of this type aim to disrupt latency without globally activating T cells and then specifically kill the virus-producing cells via immune clearance mechanisms, perhaps induced by an anti-HIV therapeutic vaccine [11]. In vitro studies suggest that protein kinase C [12] and histone deacetylase inhibitors [13] can shock a fraction of proviruses out of latency, and modestly encouraging results have been obtained in patients using the US Food and Drug Administration–approved histone deacetylase inhibitor vorinostat [14]. Further exploration of the use of latency-reversing agents for the eradication of HIV reservoirs, especially in combination and in the context of HSCT, is warranted.

CAN PRETRANSPLANT CONDITIONING ERADICATE HIV RESERVOIRS?

During HSCT, pretransplant conditioning is used to reduce the burden of malignant cells and create space for the engraftment of transplanted hematopoietic cells. The Berlin patient received several rounds of aggressive, high-intensity conditioning for his transplants, including myeloablative total body irradiation

HIV/AIDS • CID 2015:60 (15 January) • 293
Conditioning regimens will likely fail to eradicate all cells containing replication-competent HIV. Conceptually, GVH responses resulting from allogeneic cell transplants could be exploited to further reduce or eradicate HIV reservoirs, just as graft-vs-leukemia (GVL) responses can reduce or eradicate leukemic cells [17]. Minimizing the morbid consequences of GVHD while stimulating graft-vs-viral reservoir (GVVR) responses will be a significant obstacle to using HSCT in HIV cure regimens.

CD34-expressing stem cells together with mature donor T cells and natural killer (NK) cells are a source of immune system replenishment following HSCT [18]. GVHR results from alloreactive T cells encountering recipient cells presenting alloantigens, leading to the destruction of epithelial and endothelial surfaces, as well as hematopoietic cells. Dividing cells, including tissue-regenerating stem cells, are the primary targets. The severity of GVHD reflects the balance between immune damage and tissue repair, with the extent of damage dictated primarily by the number and nature of mismatched antigens [19, 20]. Other factors influencing GVHD include the patient’s age, microbiota, nutritional status, and genetics (e.g., DNA repair deficiencies, cytokine polymorphisms), as well as the extent of organ damage before or during the transplant, dose of T cells in the graft, and frequency of regulatory T cells [19–22].

Depleting T cells from grafts prior to transplantation can prevent GVHD; however, these grafts are deficient in desirable GVL responses, engraft less efficiently, and predispose to high rates of infection [22, 23]. One potential solution is to transplant T-cell–depleted grafts and then use donor lymphocyte infusions (DLIs) to selectively repopulate desirable T-cell populations [23]. Other potential approaches include transplantation of in vitro expanded donor HIV-specific T cells or T cells transduced with chimeric antigen receptors that recognize HIV [23]. Unfortunately, HIV’s extensive capacity to mutate may enable escape from these approaches. Alternatively, DLI with cytokine-induced NK cells may provide GVL and GVVR responses with minimal GVHD; NK cell–based transplantation approaches may also be advantageous as these cells are not susceptible to HIV infection. Still another possibility is the selective depletion of alloreactive T cells, either ex vivo or in vivo [24, 25]. This approach has been used successfully to reduce GVHD during HSCT for sickle cell disease [26].

In contrast to transplanted allogeneic T cells, which cause GVHD by damaging epithelia and endothelia, transplanted NK cells only target recipient hematopoietic cells [27]. Thus, NK cells assist GVL responses by eradicating leukemic cells without contributing to GVHD [27]. It is plausible that allogeneic NK cell responses might similarly produce GVVR responses following HSCT.

NK cell activation is regulated in part by interactions between human leukocyte antigen (HLA) molecules and killer cell immunoglobulin-like receptors (KIRs), for which there are activating and inhibitory isoforms, as well as allelic diversity [27]. NK cells expressing the KIR3DL1 receptor can kill allogeneic cells infected with HIV [28], and certain KIR3DL1 alleles protect against HIV when combined with specific HLA-Bw4 alleles [29]. This combination likely activates NK cells to kill HIV-producing cells. The selection of HSCT donors with favorable NK cell genetics might prevent the establishment of new reservoirs posttransplant by enabling NK cells to kill donor hematopoietic cells that become infected with HIV, while promoting full donor chimerism and, in that process, eradicating HIV reservoirs [20, 27, 30]. HLA-C genotypes should also be taken into account when choosing allogeneic donors for HIV-infected patients, as high-level expression of HLA-C protects against HIV [31] but contributes to GVHR when mismatched [20].
Berlin patient was matched for HLA alleles but mismatched for KIRs. It is not known whether KIR immunogenetics contributed to his elimination of HIV, but the available data suggest that these factors should be considered in future HSCT regimens for HIV-infected individuals. Recent advances in NHP genotyping may allow these issues to be investigated in NHP models [32] as well as in human studies.

Because many patients appear to achieve full donor chimerism without developing overt GVHD, it should be possible to safely use GVH responses to exert significant GVVR activity in HIV-infected patients requiring HSCT for malignancies. Interestingly, vorinostat, which can disrupt HIV latency, has been shown to reduce GVHD in HSCT recipients without having deleterious effects on engraftment or malignancy relapse [33], suggesting that it might suppress GVHD without impeding GVVR. Although significant clinical advances have reduced the frequency of severe GVHD, there is still a 7% transplant-associated mortality rate in patients with malignancies, a rate that far exceeds what would be acceptable for healthy HIV-infected patients on cART.

**CAN WE PROTECT TRANSPLANTED CELLS FROM HIV INFECTION?**

As discussed, it may be possible to use conditioning regimens and GVVR responses to reduce or eliminate HIV reservoirs. In addition, it will be critical to prevent any transplanted cells from becoming infected and establishing new reservoirs. Where- as maintaining patients on cART throughout the transplant period should theoretically prevent infection of donor cells, concerns about drug–drug interactions often lead physicians to discontinue antiretrovirals during transplantation [6, 34]. In addition, patients undergoing transplantation often develop nausea and become intolerant of oral medications. Although efforts have been made to identify more tolerable cART regimens [34], even the most effective cART regimens may fail to adequately penetrate all tissues and fully suppress HIV replication [35, 36].

Viremic spikes have been observed posttransplant in NHP models, even when cART was meticulously maintained (Leslie Kean, unpublished data). A likely explanation is that some latently infected T cells become activated during transplantation, thereby stimulating virus production, as documented in murine models of latent retrovirus infection. Indeed, the viral outgrowth assay used to quantify replication-competent HIV in peripheral blood relies upon the in vitro activation of CD4 T cells through a mixed lymphocyte reaction to stimulate viral replication. These observations suggest that HIV reactivation will accompany transplant-associated GVH responses and may not be suppressed adequately by cART. Thus, preventing infection of transplanted grafts requires novel approaches.

One approach to preventing infection posttransplant is to use grafts that intrinsically resist HIV infection. Intrinsic resistance was almost certainly a key element of the Berlin patient’s cure. CCR5 is a coreceptor for cellular entry by HIV. Individuals who are homozygous for the nonfunctional CCR5-D32 allele are highly resistant to HIV infection [7]. The Berlin patient’s transplant donor was homozygous for the CCR5-D32 allele. In addition, the Berlin patient himself is a CCR5-D32 heterozygote. Heterozygosity for CCR5-D32 is associated with delayed progression of HIV disease [37] and recent data suggest that CCR5-D32 heterozygotes have lower HIV reservoirs (Steven Deeks, unpublished data).

Like the Berlin patient, both Boston patients were CCR5-D32 heterozygotes. However, they received transplants from CCR5 wild-type donors. Thus, their relapses may have resulted from posttransplant infection of donor cells, possibly due to GVH-induced activation of latently infected memory T cells leading to HIV replication.

Only 1% of donors of European descent are CCR5-D32 homozygotes, and the frequency is even lower in donors of African descent. Thus, it will be difficult to identify well-matched homozygous D32 transplant donors for many patients with HIV. Also, some HIV variants can bypass CCR5 and use CXCR4 to infect T cells. Accordingly, it will be important to develop other approaches to protecting grafts from HIV infection. One possible solution is to supply patients with genetically modified cells that resist HIV infection. A number of genetic engineering strategies are being explored in NHP models and humans with promising results [38, 39].

**ADDITIONAL CHALLENGES AND RESEARCH QUESTIONS**

A number of questions were highlighted by the workshop (Table 1), and it remains to be seen whether we can exploit...
GVH-based approaches to cure HIV in a safe, scalable, and widely applicable manner. One major obstacle is the need for better assays to determine when cART can be discontinued. Currently available assays for HIV are inadequate to detect all latently infected cells. Likewise, the PCR-based assays commonly used to measure donor chimerism after HSCT cannot distinguish whether low-level positivity reflects residual hematopoietic cells potentially bearing HIV genomes or harmless nonhematopoietic cells contaminating blood at low frequencies. It will also be challenging to adequately sample every tissue that may harbor residual recipient hematopoietic cells potentially carrying latent HIV. Moreover, a subset of HIV sequences found in the blood of patients on cART does not correspond to virus found in circulating T cells, suggesting these sequences may derive from yet to be identified reservoirs [40].

Based on current understanding, a prototype cure strategy for HIV-infected patients requiring HSCT for another indication (eg, malignancy) would leverage pretransplant conditioning to deplete HIV reservoirs and then enlist GVVR responses to eradicate residual reservoirs while preventing infection of transplanted cells by maintaining cART throughout the procedure and/or using HIV-resistant grafts. Should such an approach prove effective, extending it more generally to the HIV-infected community will still require substantial reductions in HSCT-associated morbidity and better assays for residual reservoirs of latently infected cells. Collaboration between the HIV and transplantation research communities will be necessary to overcome these significant challenges.

More detailed summaries of the workshop presentations are provided as Supplementary Data.

**Supplementary Data**

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

**Acknowledgments.** We thank the workshop organizers, speakers, and session moderators: Richard Ambrinder, John Barrett, Catherine Bollard, Nancy Bridges, Nelson Chao, Steven Deeks, Carl Diefenbach, Michele Di Mascio, Christine Durand, Sean Emery, Anthony Fauci, Daniel Fowler, Juan Gea-Banacloche, Ronald Gress, Charles Hackett, Timothy Henrich, Katherine Hsu, Robert Jeng, Leslie Keen, Hans-Peter Kiem, Robert Korngold, Kristy Kraemer, Daniel Kuritzkes, Clifford Lane, Jeffrey Lifson, John Mellors, Everett Meyer, Sophie Paczysny, Deborah Persaud, Effie Petersdorf, Pavan Reddy, Warren Shlomchik, John Tisdale, Lis Welniaik, and Ann Woolfrey.

**Potential conflicts of interest.** All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


