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Hematopoietic cell transplantation and HIV cure: where we are and what next?

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The report of the so-called Berlin patient cured of HIV with hematopoietic stem cell transplantation and a few other studies raised tremendous hope, excitement, and curiosity in the field. The National Heart, Lung and Blood Institute of the National Institutes of Health convened a Working Group to address emerging heart, lung, and blood research priorities related to HIV infection. Hematopoietic cells could contribute to HIV cure through allogeneic or autologous transplantation of naturally occurring or engineered cells with anti-HIV moieties. Protection of central memory T cells from HIV infection could be a critical determinant of achieving a functional cure. HIV cure can only be achieved if the virus is eradicated from reservoirs in resting T cells and possibly other hematopoietic cells. The Working Group recommended multidisciplinary efforts leveraging HIV and cell therapy expertise to answer the critical need to support research toward an HIV cure. (*Blood*. 2013;122(18):3111-3115)

Background

Highly active antiretroviral therapy (HAART) or combination antiretroviral therapy (cART) has been so effective that HIV infection has now been transformed from a deadly disease to a chronic infection. Nevertheless, such therapies do not eradicate the virus, which persists in reservoirs that are not yet fully understood.[1,2] Consequently, patients must continue lifelong therapy, which has adverse effects; furthermore, it is costly and therefore not sustainable for people living in the parts of the world hardest hit by the epidemic. Thus, current research is directed at discovering new strategies toward a cure for HIV, including transplantation of engineered hematopoietic stem/progenitor cells and T cells as well as naturally occurring hematopoietic stem/progenitor cells that give rise to progeny resistant to HIV.

The report of a single patient cured of HIV with hematopoietic stem cell (HSC) transplant has raised tremendous hope, excitement, and curiosity in the field. The patient had HIV infection and leukemia and was transplanted in Berlin in 2007 using HSCs from a donor whose cells lacked the functional CCR5 coreceptor required for HIV to infect cells. Following the transplant, his antiretroviral therapy was stopped and his blood and various biopsy specimens showed no detectable HIV,[3,4] suggesting that he might be cured. However, it is not certain how functional cure was achieved with the "Berlin" patient: it could be attributable to some combination of the transplant conditioning regimen, the anti–T-cell chemotherapy, graft-versus-host effect, and/or other factors in addition to the lack of a functional CCR5 coreceptor.[5] In addition to the Berlin patient, there have been early-stage clinical studies of engineered human progenitor cells and T cells expressing anti-HIV moieties, including CCR5-inactivated T cells.[6-15] These studies demonstrated the safety and feasibility of such therapies, but their efficacy is yet to be evaluated.

NHLBI AIDS Working Group September 2012

In this context, the National Heart, Lung, and Blood Institute (NHLBI) convened a Working Group on September 6-7, 2012, in Bethesda, MD to address emerging research priorities in noninfectious HIV-related heart, lung, and blood diseases. The primary aim of the meeting was to elicit recommendations that would inform the future goals and strategic plan of the NHLBI AIDS program through multidisciplinary sessions and organ-specific breakout sessions. The 2 blood sessions addressed HIV-related anemia and the role of hematopoietic stem and progenitor cells both as potential reservoirs and as potential cures for the disease. Working Group participants were asked to identify the highest priority research gaps within these areas and recommend future research strategies to address those gaps. The Executive Summary of the Working Group is available at http://www.nhlbi.nih.gov/meetings/workshops/AIDSworking.htm. The following key research questions were identified during deliberations:

1. Does anemia occur more commonly among successfully treated HIV-infected populations and does it have an impact on morbidity and mortality?
2. What is the pathogenesis of HIV-related cytopenias?
3. What role do hematopoietic stem/progenitor cells and the stem cell niche (hematopoietic stem cells) play in HIV infection as potential reservoirs and/or cure?

Research was proposed to address these key questions, including assessment of the prevalence and pathophysiology of cytopenias, and associations of cytopenias with other markers of noninfectious complications of HIV and with changes in marrow stem cells and the marrow microenvironment. A major discussion focused on

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determining the potential therapeutic application of cellular therapies directed against HIV through autologous and allogeneic stem cell and T-cell therapies for potential eradication and cure of HIV infection and understanding the mechanism behind the cure in the Berlin patient. The need for improving processes for purification, production and expansion, and genetic modification of stem and T cells to allow for effective anti-HIV effects was identified. There was a call for evaluation of the role of allogeneic mismatch and reactivity in graft-versus-host disease (GVHD) and graft-versus-HIV effects, with the ultimate goal of using transplant modalities to control or cure HIV in the nonmalignant setting. For cytopenias, the Working Group recommended interrogation of existing cohort data, prospective assessment in interventional trials, and the conduct of interdisciplinary pathogenesis studies to better understand the mechanisms of cytopenias in HIV disease and related multiorgan disease (ie, heart, lung). For stem cells and cellular therapies, the Working Group recommended pathogenesis studies including xenograft assessment in suitable animal models, broad-based assessment of the role of stem cell transplantation and cellular therapies in HIV cure, and the need to study approaches for stem cell expansion. The need for scientific review of proposals by special emphasis panels was discussed and the Working Group thought that multidisciplinary efforts should be encouraged. This *Blood* Forum aims to place the recommendations on cellular therapies in the context of the current state of the art.

Current state of the art

The discussions of the NHLBI AIDS Working Group and review of the literature suggest several strategies for the use of hematopoietic cells and other insights to cure HIV that are summarized here.

Transplantation of naturally occurring CCR5 defective hematopoietic stem/progenitor cells

Modeled in the "Berlin" patient, a transplantation of naturally occurring CCR5 defective hematopoietic stem/progenitor cells could represent a real option for certain HIV-infected patients. The protocol of the Blood and Marrow Transplant Clinical Trial Network (BMT CTN) for patients with chemotherapy-sensitive hematologic malignancies and coincident HIV infection suggests that where possible, one should identify donors homozygous for the Δ32 mutation for CCR5 (http://clinicaltrials.gov/show/NCT01410344). However, the limited availability of homozygous CCR5Δ32 coupled with the need for close HLA matching limits the feasibility of this approach. Because cord blood transplantations require significantly less stringent HLA matching, efforts have been made to identify cord blood units from homozygous CCR5Δ32 donors for HIV cure. Hematopoietic cell transplantation was performed with cells from one such unit, which was identified as homozygous CCR5Δ32 after being released for transplant. The recipient was not infected with HIV. In vitro studies posttransplantation indicated that the patient's peripheral blood mononuclear cells were resistant to HIV infection.[16] Most recently, a 12-year-old boy born with HIV and with acute lymphoblastic leukemia was transplanted with a cord blood unit of homozygous CCR5Δ32 (http://www.poz.com/articles/761_23834.shtml) at the University of Minnesota. The plan was to temporarily stop HIV drugs once HIV was undetectable in blood and tissues in order to test whether a cure for the virus was achieved (http://www1.umn.edu/news/features/2013/UR_CONTENT_440332.html). Unfortunately, the patient died of GVHD complications posttransplant before it could be evaluated if a cure for the virus was achieved (http://kstp.com/article/stories/s3095974.shtml, last accessed on August 21, 2013). In addition to this risk of GVHD that is associated with all allogeneic HSC transplants, a sufficiently high number of transplanted HSCs (a minimum of approximately 1.5-2.5 $\times$ 10^7/kg precryopreserved total nucleated cell dose) is sometimes difficult to obtain from cord blood samples. Consequently, many centers use 2 cords to ensure adequate stem cell support. Thus, it would be beneficial to develop methods to increase the number of HIV-resistant HSCs that can be obtained from cord blood samples as well as strategies to increase the efficiency of homing/engraftment of cord blood stem cells.[17,18] In combined haploidentical and cord blood transplantation, a cell dose of as low as 1 $\times$ 10^7/kg cell dose may be adequate.[19] It has been reported that this procedure largely overcomes the problem of delayed engraftment through transient engraftment of adult HSCs and results in predictable engraftment of cord blood stem cells with the desired characteristics.[20] It was further suggested that finding an adequately HLA-matched cord blood unit is more critical than finding a unit with an adequate cell dose.[20] In view of the increasing inventory of cord blood units from homozygous CCR5Δ32 donors, there is a reasonable probability of finding an adequately matched unit for a patient of HIV infection who is in need of a transplant.[16]

Autologous or allogeneic transplant of engineered hematopoietic stem/progenitor cells or T cells

With CCR5 knocked out and/or other anti-HIV moieties, autologous or allogeneic transplant of engineered hematopoietic stem/progenitor cells or T cells have been and are being actively pursued as potential cures for HIV infection. These include a phase 1 gene transfer clinical study to introduce a potential anti-HIV gene therapeutic into hematopoietic progenitor cells,[6] a phase 2 cell-delivered gene transfer trial of a tat-vpr–specific anti-HIV ribozyme (OZ1) or placebo delivered in autologous $CD34^+$ hematopoietic progenitor cells,[8] transplantation of gene-modified hematopoietic progenitor cells expressing 3 RNA-based anti-HIV moieties (tat/rev short hairpin RNA, TAR decoy, and CCR5 ribozyme),[9] a phase 1 study of anti–HIV-1 tat ribozyme-transduced $CD4^+$ T lymphocytes,[7] and 2 phase 1 trials of autologous T cells genetically modified at the CCR5 gene by zinc finger nuclease (SB-728-T).[13] These clinical studies have been summarized in previous reviews.[5,10-12] Additional results from 1 of the 2 trials with SB-728-T suggested that increased CD4 counts following SB-728-T infusion may be due to the durable maintenance of CCR5-modified central memory CD4 (T_{CM}) cells that potentially enhanced survival of endogenous T_{CM} cells, a surrogate marker of slow disease progression.[21] There are other ongoing studies. One study is determining the safety and feasibility of lentivirus vector rHIV7-shI-TAR-CCR5RZ–transduced hematopoietic progenitor cells in the setting of autologous hematopoietic cell transplantation to treat AIDS-related lymphoma (http://clinicaltrials.gov/show/NCT00569985). Another trial will study genetically modified peripheral blood stem cell transplantation to treat HIV-associated non-Hodgkin or Hodgkin lymphoma (http://clinicaltrials.gov/show/NCT01769911). In another planned study, patients will be treated with autologous $CD34^+$ cells transduced with an antiviral vector (M87o) encoding for the HIV-1–entry inhibitor peptide C46 (http://clinicaltrials.gov/show/NCT00858793). An early phase research study is exploring whether an experimental gene transfer, LVsh5/C46 (also known as Cal-1), is safe and whether it can protect the immune system from the effects of HIV without the use of

antiretroviral drugs. Cal-1 is an experimental gene transfer agent designed to inhibit HIV infection through 2 active parts: (1) removing CCR5 from bone marrow and white blood cells, and (2) producing C46 on bone marrow and white blood cells (http://clinicaltrials.gov/ct2/show/NCT01734850). It has been shown that autologous transplantation of HSCs expressing a fusion inhibitor in macaques (mC46) resulted in a positive selection of gene-modified $CD4^+$ T cells in peripheral blood, gastrointestinal tract, and lymph nodes, accounting for >90% of the total $CD4^+$ T-cell population following simian/human immunodeficiency virus challenge. The mC46 macaques also maintained high frequencies of simian/HIV-specific, gene-modified $CD4^+$ T cells, an increase in non-modified $CD4^+$ T cells, enhanced cytotoxic T lymphocyte function, and antibody responses. The results demonstrated the possible benefits of genetically modified, autologous HSCs to treat HIV-infected patients.[22] Kiem et al[14] in their recent review outlined the steps necessary to realize the goal of HSC-based gene therapy for HIV disease: (1) HSCs must be identified and purified (and/or expanded) in numbers sufficient to provide a benefit in both adults and children; (2) methods must be devised to efficiently and stably introduce novel gene functions into HSCs; (3) the selected gene functions must be shown to confer HIV resistance in progeny T cells and myeloid cells; (4) the gene-modified cells must be introduced into the patient safely and efficiently; and (5) clinical trials must be designed to convincingly demonstrate efficacy.[14] Furthermore, additional approaches are being explored in preclinical studies. For example, a recent study used ZFNs to insert a cocktail of anti-HIV restriction factors into the CCR5 locus in a T-cell reporter line, knocking out the CCR5 gene in the process.[23] In their review, Kiem et al also outline various host and viral targets that could be exploited for HIV cure, as well as certain primate host restriction factors that may be explored to prevent HIV infection of cells and may represent potential candidates for gene therapy.[14] It is possible that to be effective any gene therapy approach may need to be combinatorial in nature, simultaneously targeting multiple stages of the viral life cycle.[24,25]

Allogeneic hematopoietic stem/progenitor cell transplant without gene transfer but with cART may represent another approach toward HIV cure

Henrich et al[26] reported long-term reduction in peripheral blood HIV-1 reservoirs following reduced-intensity conditioning allogeneic stem cell transplantation in 2 HIV-positive individuals, both of whom were heterozygous for the CCR5Δ32 mutation. In-depth analyses of the HIV-1 reservoir size in peripheral blood, coreceptor use, and specific antibody responses on samples obtained before and up to 3.5 years after hematopoietic stem cell transplantation (HSCT) revealed that although HIV-1 DNA was detected in peripheral blood mononuclear cells before and 2 to 3 months after HSCT, HIV-1 DNA and RNA were undetectable in peripheral blood mononuclear cells, $CD4^+$ T cells, or plasma up to 21 and 42 months after HSCT. The loss of detectable HIV-1 correlated temporally with full donor chimerism, development of GVHD, and decreases in HIV-specific antibody levels. The ability of donor cells to engraft without evidence of ongoing HIV-1 infection suggests that HIV-1 replication may be fully suppressed during cART and does not contribute to maintenance of viral reservoirs in peripheral blood in the patients. HSCTs with wild-type-CCR5(+) donor cells can lead to a sustained reduction in the size of the peripheral reservoir of HIV-1. The authors concluded that continuous administration of effective antiretroviral therapy protected the donor cells from becoming HIV infected as they eliminated and replaced the patients' immune cells, effectively clearing the virus from their blood lymphocytes. Tissue sampling and analytic treatment interruption are needed to assess fully the extent of HIV-1 reservoir reduction following allogeneic stem cell transplantation. (AIDS 2012 Press Releases, http://www.aids2012.org/Default.aspx?pageId=384). Durand and Ambinder commented in their recent review that the report supports the hypothesis that allogeneic HSCT and ART might cure HIV-1 infection.[15] At the most recent 7th IAS Conference on HIV Pathogenesis, Treatment and Prevention held on June 30 to July 3, 2013, in Kuala Lumpur, Malaysia, Henrich et al further reported that the 2 patients have stopped taking HIV drugs but have remained free of HIV for almost 4 months and almost 2 months, respectively (http://www.ias2013.org/Default.aspx?pageId=635; http://www.nature.com/news/stem-cell-transplants-may-purge-hiv-1.13297).

Early cART may prevent HIV from establishing a reservoir

A 2-year-old child born with HIV infection and treated with antiretroviral drugs beginning in the first days of life lost evidence of detectable levels of virus by conventional testing despite cessation of HIV medication for 10 months.[27] An editorial published in *BMJ* cautioned that many experts are uncertain as to whether the baby had been truly infected, because this may have been a case of prevention of transmission rather than cure.[28] Another study of 5 perinatally HIV-infected youths (median age, 16 years) who began ART at a median of 2 months of age suggested that long-term control of HIV replication following early ART in infancy reduces resting $CD4^+$ T cells bearing proviral DNA, replication-competent virus, and residual plasma viremia, with an absence or a reduction in HIV-specific immune responses. The authors suggest that early treatment of perinatally infected youths markedly curtails HIV reservoirs, making them prime candidates for interventions to achieve functional cure or eradication.[29] Although encouraging, longer periods of follow-up and additional studies are needed to verify these findings.

Protection of central memory T cells (T_{CM}) may shed light on the underlying mechanism for HIV cure

New findings about how a subset of $CD4^+$ white blood cells invaded by HIV may control the course of the disease promise to have a profound impact on the field. These "central memory cells" might even help explain the underlying mechanism behind the child's apparent cure, as stated above.[30] One study conducted in Thailand identified people shortly after becoming infected with HIV and encouraged them to start antiretroviral treatment immediately. They showed that early treatment prevented seeding of latent reservoirs in long-lived central memory T cells.[30,31] Another study examined "elite controllers," who naturally control HIV better than others and protect their T_{CM} cells without the help of antiretrovirals, and found their T_{CM} cells downregulate expression of CCR5 coreceptor and were less permissive to HIV infection. Conversely, people whose immune systems do not rebound even though antiretrovirals control their infections have functionally impaired T_{CM}.[32] As stated earlier, updated findings from a phase 1 clinical trial of SB-728-T also suggest that T_{CM} could be the critical component for sustained CD4 reconstitution in HIV subjects receiving SB-728-T.[21] These studies underscore the role of central memory cells in finding an HIV cure and suggest that protection of central memory cells from HIV might be a critical determinant of achieving a functional cure.

Hematopoietic cells and HIV reservoirs

Finally, hematopoietic cells and HIV reservoirs could be central to any strategies toward HIV cure. Current evidence suggests that cART (or HAART) effectively halts ongoing viral replication, whereas residual viremia originates from stable reservoirs rather than ongoing replication. This is important because this realization has led to a shift in the HIV-1 treatment field toward the eradication of reservoirs. Current consensus suggests that HIV-1 persists only in cells of hematopoietic origin, and latently infected resting $CD4^+$ T cells are clearly recognized as HIV reservoirs.[2] Indeed, Durand et al reported that 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 with undetectable HIV viral load.[33] Another recent study also reported that hematopoietic precursor cells isolated from patients on long-term suppressive HIV therapy do not contain HIV-1 DNA.[34] However, Carter and colleagues reported that HIV-1 infects multipotent progenitor cells, causing cell death and establishing latent cellular reservoirs.[35] These investigators also reported that HIV-1 utilizes the CXCR4 chemokine receptor to infect multipotent hematopoietic stem and progenitor cells.[36] In their latest report, they indicate that HIV-1 may establish a latent infection in all subsets of hematopoietic progenitor cells they examined, including immature populations of HSCs and multipotent progenitors. Many factors associated with HIV infection or study procedures may account for differences in findings between studies, and additional studies are needed to more fully understand HIV reservoirs.[37] Other potential reservoirs include macrophages and microglial cells.[2] If microglial cells constitute a reservoir of latent HIV infection in the central nervous system, then the kinetics of their replacement by cells of donor origin could be an important determinant of time to HIV eradication following allogeneic HSCT.

Future directions

It is apparent that hematopoietic cells could contribute to HIV cure in several ways, including transplant of CCR5-defective cells (especially those derived from cord blood), autologous and allogeneic transplant of engineered cells with CCR5 knocked out and/or other anti-HIV moieties, allogeneic transplant without gene transfer but with cART, early cART to prevent the establishment of HIV reservoirs in hematopoietic cells, and protection of T_{CM} from HIV infection. Regardless of the role of hematopoietic stem/progenitor cells as reservoirs, HIV cure can only be achieved if the virus is eradicated or eliminated from hematopoietic cells, and thus HSCT may play a critical role in HIV cure through replacement of HIV-infected hematopoietic cells. Alternatively, protection of T_{CM} could also help achieve a cure.

Following the NHLBI AIDS Working Group meeting in September 2012, discussions have continued on research priorities to support innovative approaches beyond HAART toward a cure for HIV-1. Approaches of interest include cell therapies (including those based on HSCs), nontraditional antiviral strategies, new delivery systems, and novel gene therapy approaches. Multidisciplinary efforts that leverage HIV expertise with cell and gene research experience may foster critical research toward an HIV cure. These priorities are consistent with NHLBI's interest in supporting research on HSCs and cell therapy and are timely given the progress in clinical transplant therapy. The current NIH AIDS Strategic Plan identified "research toward a cure" as a major priority area. By building on and synergizing research experience and HIV and cell therapy research expertise, the time has come to accelerate research on a topic of tremendous public health potential.

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Authorship

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A list of additional members of the NHLBI AIDS Blood Session Working Group participants appears in "Appendix."

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Appendix

The NHLBI AIDS Blood Session Working Group participants included Nancy Berliner (chair), Daniel Kuritzkes (cochair), and (in alphabetical order) Charles Abrams, Richard Ambinder, Michael Busch, Steven Deeks, Michelle Floris-Moore, Hans-Peter Kiem, Scott Kitchen, Michael Lederman, Nina Lin, Mohandas Narla, Satish Pillai, Margaret Ragni, Heather Ribaudo, and David Scadden. NHLBI representatives included Keith Hoots (director of the Division of Blood Diseases and Resources) and Donna Dimichele (deputy director of the Division of Blood Diseases and Resources), as well as Simone Glynn and Shimian Zou (NHLBI AIDS Blood Team leaders).

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