Cord Blood Transplantation for Cure of HIV Infections

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SUMMARY
HIV infection has not been cured by antiretroviral drugs or gene therapy, but it has been cured by a hematopoietic cell transplantation (HCT) that was performed for a patient with acute myeloid leukemia and HIV infection using peripheral blood stem cells from an adult donor homozygous for CCR5-Δ32 (CCRS5-Δ32/Δ32). HIV has remained undetectable more than 6 years after discontinuation of antiretroviral therapy. However, this approach cannot be readily generalized because of the low prevalence of the CCR5-Δ32 allele and the need for a very close human leukocyte antigen (HLA) match between adult donors and recipients, as when bone marrow or peripheral blood stem cell transplants are performed. In contrast, cord blood (CB) transplants require less stringent HLA matching. CB units are being screened to develop an inventory of cryopreserved homozygous CCR5-Δ32 units available for HCT. One hundred eighty homozygous CCR5-Δ32 units have been identified, and 300 units are projected to provide for white pediatric patients a 73.6% probability of finding an adequately HLA-matched unit with a minimal cell dose of \( \geq 2.5 \times 10^7 \) total nucleated cells (TNC) per kilogram and for white adults a 27.9% probability. With a minimal cell dose requirement of \( \geq 1 \times 10^7 \) TNC per kilogram, the corresponding projected probabilities are 85.6% and 82.1%. CB transplantation does not require as stringent an HLA match between donor and recipient as bone marrow or peripheral blood HCTs, and HCT using cord bloods from donors homozygous for CCR5-Δ32 is, at the present time, the only feasible means of treatment of reasonable numbers of patients who are infected with HIV.

INTRODUCTION
More than 40 years after the onset of the HIV pandemic, the disease has not been cured using highly active antiretroviral treatment, by gene therapy, or by hematopoietic cell transplantation (HCT) using cells from the general pool of stem cell donors. The most important reason for natural protection against HIV transmission is a mutation in the CCR5 gene leading to a 32-base pair deletion (CCRS5-Δ32) [1–3]. In February 2007, Hütter et al. [4] performed an HCT in a patient with acute myelogenous leukemia and HIV infection using stem cells obtained from the peripheral blood of a donor who was homozygous for the CCR5-Δ32 deletion. More than 6 years after the transplant, the patient (now known as the “Berlin Patient”) does not require antiretroviral therapy (personal communication, Timothy Brown, the “Berlin Patient,” July 2013). Laboratory tests have indicated that there has been no detectable HIV in the bloodstream as indicated by analysis of viral RNA and cellular proviral DNA. In addition, the CD4+ T cells have returned to a normal range. Therefore, investigators have concluded that transplantation of homozygous CCR5-Δ32 stem cells has led to a functional cure or a “sterilizing cure” of HIV infection [5]. Deeks and McCune [6] have commented that “the HIV research community is hesitant to use the word ‘cure,’ but this single case could very well be the first example to fit the bill.”

One would expect that such a successful procedure would be performed repeatedly in other HIV-infected patients. Obstacles to the frequent use of HCT for the cure of HIV are that people who are homozygous for the CCR5-Δ32 allele are quite unusual (<1% of whites, and a much lower percentage in other ethnic groups), and most patients in need of an HCT have only a small number of potential donors from among registries of adult volunteer donors.

Moreover, when the donor stem cells are obtained from adults, as in bone marrow or peripheral blood stem cell transplants, a very close human leukocyte antigen (HLA) match is required between donors and recipients for eight of eight or seven of eight high-resolution alleles at four loci (A, B, C, DRB1) [7]. Thus, finding a donor who has a very close HLA match to a patient in need of a transplant and who is also homozygous for the CCR5-Δ32 allele is extremely difficult and will only rarely be possible. Indeed, Hütter and Thiel [8] indicate that they have had requests to perform HCT in additional HIV-infected patients. However, none of the available adult donors had the homozygous CCR5-Δ32 deletion. Accordingly, no further HCTs of patients with HIV infection using homozygous CCR5-Δ32 stem cells from adult donors have been performed [8].

In marked contrast is the fact that using stem cells from umbilical cord blood for HCT provides a major advantage in that much less stringent HLA matching between donor and recipient is required.

Indeed, acceptable HLA-matched units include those that are matched at four of six, five of six, or six of six alleles at three loci using low-resolution testing at the A and B loci and high-resolution testing at the DRB1 locus, allowing two mismatches at the same locus for four of six matching. Therefore, one hypothesis is that an inventory of cryopreserved umbilical cord blood units that are homozygous for the CCR5-Δ32 allele will provide a significantly improved probability of finding an appropriately HLA-matched homozygous CCR5-Δ32 donor for HCT of a patient with HIV infection.

METHODS
To test this hypothesis, an inventory of cryopreserved homozygous CCR5-Δ32 cord blood units is being developed to be used for HCT of
appropriate patients. Samples have been tested from approximately 20,000 cryopreserved cord blood units obtained primarily from whites, and 180 homozygous CCR5-Δ32 units have been identified, for an incidence of approximately 0.8%. Testing an additional 15,000 samples from whites is expected to increase the special inventory to approximately 300 units. Development of a 300-unit special inventory is eminently feasible since, according to the estimates of Gonzales et al. [9] there are approximately 400,000 cord blood units cryopreserved around the globe, and among them are 2,000–4,000 homozygous CCR5-Δ32 units.

The testing of large numbers of samples requires the collaboration of multiple cord blood banks since no single bank has enough units. Collaborating cord blood banks are those of St. Louis, Missouri; Duke University; University of Colorado; MD Anderson Cancer Center; Sydney, Australia; and Barcelona, Spain. CCR5 genotype analysis is performed on DNA extracted from cord blood using a polymerase chain reaction-based assay for homozygosity of the CCR5-Δ32 base pair deletion.

Biostatematicians at the National Marrow Donor Program (NMDP) have developed estimates regarding the probability of being able to provide an adequately HLA-matched cord blood unit with an adequate cell dose for a patient from among an inventory of 300 homozygous CCR5-Δ32 units. These projections indicate that such an inventory would provide for whites an adequately HLA-matched cord blood unit (at least four of six alleles) with an adequate cell dose for 73.6% of pediatric patients and 27.9% of adult patients, considering a minimal cell dose requirement of $\approx 2.5 \times 10^7$ total nucleated cells (TNC) per kilogram.

Since Liu et al. [10] have reported that a cord blood cell dose as low as $1 \times 10^7$ TNC per kilogram of body weight is adequate for cord blood transplants done in association with a haploidentical transplant, projections were also made regarding the probability of finding an adequately matched unit in a 300-unit inventory using this as the minimum necessary dose. Such projections indicate a probability of finding an adequately HLA-matched cord blood unit with a cell dose requirement of $\approx 1 \times 10^7$ TNC per kilogram of 85.6% for white pediatric patients and 82.1% for white adult patients. For members of minority ethnic groups, the projected probabilities using a minimal necessary cell dose of $1 \times 10^7$ TNC per kilogram are significantly lower (e.g., 34.1% for black children, 52.5% for Mexican American children, and 15.7% for Chinese American children).

**Discussion**

The use of combined haploidentical and cord blood transplants [10] provides important, and probably essential, advantages when considering the use of HCT to cure HIV in adults. Most adults require a cord blood cell dose that cannot be satisfied using a single cord blood unit. For most cord blood transplants in adults, this problem is solved by using double cord blood transplants. However, finding two adequately matched units for a patient from an inventory of 300 cord blood units would be very problematic, whereas the use of a single cord blood unit combined with a haploidentical transplant is much more feasible. Furthermore, in such transplants, engraftment is rapid, and chimerism studies have shown that cells from the cord blood unit are almost always the only ones present several months post-transplant [10].

The most obvious patient population for the transplantation of homozygous CCR5-Δ32 cord blood units is that group of patients who are in need of an HCT for a hematologic malignancy or other indication and are also infected with HIV. The major risks associated with this approach are those related to transplant-related morbidity and mortality. Since HCT is indicated for the underlying disorder, the risks associated with HCT would need to be borne by the patient in any case. Nevertheless, when selecting a homozygous CCR5-Δ32 unit to be used for transplantation of an HIV-infected patient who also has an independent indication for HCT, there may well be alternative donor units available that are not CCR5-negative and that have a higher cell dose and/or better HLA match. In such cases the transplant physician needs to discuss with the patient the risks and benefits of using a lower well-matched unit that has the potential to cure the HIV infection as well as the underlying malignancy.

It is reassuring that data are available regarding the engraftment potential of homozygous CCR5-Δ32 units. One such patient is that of Dr. Gero Hütter, described above, who was transplanted with cells from an adult donor. In addition, Petz et al. [11] describe an adult patient with acute myelogenous leukemia who was treated with a double cord blood transplant. Subsequent analysis of samples of the cord bloods demonstrated that one of the units was homozygous for CCR5-Δ32. It is well-known that in double cord blood transplants, one of the two units almost always predominates, and the other unit ultimately is not detectable in the patient. In this patient, the “winning unit” was the homozygous CCR5-Δ32 unit. Thus, not only did the homozygous CCR5-Δ32 unit engraft, but it became the dominant unit in this double cord blood HCT. Furthermore, at a time when chimerism studies indicated 100% engraftment by the CCR5-Δ32 unit, in vitro studies indicated that the patient’s peripheral blood mononuclear cells were resistant to HIV1 BAL and NL4-3 strains [11].

In addition to HCT for HIV-infected patients with a hematologic malignancy or other indication for a transplant, selected patients with AIDS should also be considered for a clinical trial using homozygous CCR5-Δ32 cord blood donors. Antiretroviral drugs have dramatically improved the prognosis for HIV-infected patients. However, anti-retroviral therapy for HIV infection needs lifelong access and strict adherence to regimens that are both expensive and associated with toxic effects. Thus, patients with AIDS who have responded poorly to antiviral regimens, have a poor prognosis, and are informed of the significant risks and the potential benefits of HCT should be allowed to participate in a clinical trial of HCT if an appropriately HLA-matched homozygous CCR5-Δ32 unit of adequate cell dose is available.

A seemingly logical approach to making HCT available to more patients with HIV is to test those who volunteer to be bone marrow/peripheral blood donors for the presence of the CCR5-Δ32 allele so as to develop a file of such adult donors in the national registry. Testing for CCR5-Δ32 could be done at the time donors are registered with the NMDP’s “Be the Match” Registry. However, this procedure is not likely to be cost-effective because many more adult units as compared with umbilical cord blood units would need to be tested in order to provide a file of homozygous CCR5-Δ32 units of a size that could provide a reasonable probability of a high-grade HLA match, as is required when HCT is done using stem cells from adults. Thus, it is more reasonable to suggest large-scale testing of newly donated and currently inventoried cord bloods for homozygous CCR5-Δ32 units.

**Conclusion**

HCT using peripheral blood stem cells from an adult donor homozygous for CCR5-Δ32 has produced a cure of an HIV-
infected person. Other therapeutic approaches, including the use of antiretroviral drugs or gene therapy, have not been successful. However, homozygous CCR5-Δ32 donors are quite unusual, and this, coupled with the fact that the use of stem cells from peripheral blood and bone marrow requires a very close HLA match between the donor and recipient, makes it impossible to frequently perform HCT using homozygous CCR5-Δ32 stem cells from adult donors for patients in need of treatment for HIV. Therefore, since HCT using cord blood does not require as stringent an HLA match, it must be recognized that HCT using cord bloods from donors homozygous for CCR5-Δ32 is, at the present time, the only feasible means of treatment of reasonably large numbers of HIV-infected patients [11].

**AUTHOR CONTRIBUTIONS**

L.P.: conception and design, collection and/or assembly of data, manuscript writing, final approval of manuscript.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

L.P. has compensated employment from StemCyte, stock options, and uncompensated honoraria.

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