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NEWS AND COMMENTARY

Autologous cell therapy for HIV

Stem cells, ribozymes and HIV

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In a recent Phase 2 clinical trial, Mitsuyasu *et al*.[1] demonstrated that the delivery of an anti-HIV ribozyme from a gammaretroviral vector integrated into hematopoietic stem cells (HSCs) offers a safe and promising gene therapy strategy for the treatment of this infectious disease. This gene therapy strategy relies on the idea that, after the *ex vivo* transduction of an anti-HIV gene into autologous CD34+ HSCs, these human immunodeficiency virus (HIV)-resistant progenitors can be re-infused into an HIV patient, where they will differentiate and expand to permanently protect against the onset of AIDS (Figure 1).[2] Although the inhibition of HIV has been considered a feasible and attractive candidate for gene therapy applications for over 20 years,[3] this is the first reported randomized, double-blind cell-delivered Phase 2 clinical trial for HIV-1.[1]

The Mitsuyasu *et al*.[1] study monitored 74 HIV-1-infected individuals for 100 weeks after infusion of transduced or control autologous HSCs. Thirty-eight of these patients were treated with HSCs carrying an integrated copy of a therapeutic, replication-incompetent, Moloney murine leukemia virus-based gammaretroviral vector (LNL6). The therapeutic vector (OZ1) expresses a hammerhead ribozyme against overlapping reading frames of the viral genes *vpr* and *tat* in unspliced and spliced viral transcripts, respectively. Although the researchers observed no significant differences in the viral load between OZ1 and control groups at any particular time point, they reported that transduced CD34+ cell therapy did not cause any apparent adverse effects and is safe for further exploration, thereby opening the doors for further trials with other gene therapy agents.

Over the course of the 100-week clinical study, Mitsuyasu *et al*.[1] observed sharp declines in the percentages of patients who maintained detectable levels of integrated DNA and mRNA from the OZ1 retroviral vector. In fact, at 20 weeks post-infusion, primary blood mononuclear cells from more than half of the treated patients had no detectable levels of OZ1 DNA or mRNA. This result is unexpected under the assumption that the OZ1-transduced HSCs successfully engrafted, differentiated and expanded into the T-cell lineage, and maintained some level of anti-HIV protection over untreated CD4+ T cells. Similar Phase I trials—including reports from the same authors[4]—have confirmed successful engraftment, differentiation and expansion of autologous HSCs transduced with anti-HIV retroviral vectors.[2] Therefore, although the HSCs may have failed to engraft under the conditions of the trial, it is also possible that the anti-HIV ribozyme failed to protect the OZ1 cells from HIV-1 infection and replication. This could be the result of mutational escape of the virus, suboptimal function of the ribozyme *in vivo* or possible shortcomings associated with gammaretroviral vectors.

This Phase 2 investigation marks an important step toward a safe, cell-delivered gene therapy for HIV. However, to further advance this promising gene therapy approach, it will be necessary to identify the particular limitations and modify the existing therapeutic strategy. First, the virus might have acquired mutations in the target region of the ribozyme that would abrogate the function of the anti-HIV agent. Mutations arising at the second or third positions of the three-nucleotide ribozyme cleavage site of spliced and unspliced viral transcripts might render the anti-HIV ribozyme ineffective. Close examination of the targeted region suggests

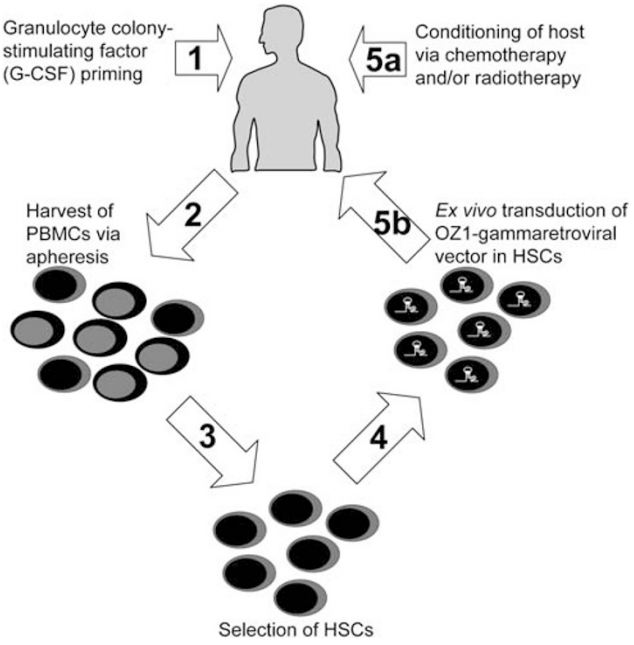

Figure 1 Cell-delivered gene therapy strategies using autologous CD34+ hematopoietic stem cells (HSCs). Human immunodeficiency virus (HIV) patients are treated with granulocyte colony-stimulating factor (G-CSF) to mobilize HSCs before collection (step 1). Primary blood mononuclear cells (PBMCs) are harvested by large-volume apheresis and HSCs are selected and purified (steps 2–3). HSCs are transduced with the anti-HIV gene therapy vector and infused back into the patient (steps 4 and 5b). Although not performed in the Mitsuyasu *et al*.[1] trial, patients may receive bone marrow conditioning by chemotherapy and/or radiotherapy to potentially increase the efficiency of engraftment (step 5a).

that mutational escape can occur with minimal detriment to either viral reading frame or viral fitness. For instance, mutation to the cleavage site would only affect the second to last amino acid in the Vpr protein, which is not essential for Vpr function.[5] Although the cleavage site occurs at nucleotide position 12 in the *tat* reading frame, silent mutations at this position would not change the coding for valine (V4), and thus escape mutations may occur with no change in Tat protein.

In addition, if the ribozyme experienced suboptimal kinetics and function *in vivo*—perhaps due to physiological conditions of Mg(2+), pH and so on[6]—the OZ1 cells would offer limited or no selective advantage over untreated cells. In some cases, the expression of the integrated ribozyme might have become silenced by heterochromatin, and consequently these transgenic cells would be unprotected from HIV infection and replication. Alternatively, as gammaretroviruses often integrate near the transcriptional start sites of genes,[7] significant fractions of OZ1-treated cells may have induced the overexpression of genes that promote the accelerated death of protected cells. Although the above scenarios are plausible, the failure to detect expansion of the transduced cells in peripheral T lymphocytes suggests poor engraftment of the transduced HSCs, perhaps owing to lack of conditioning before HSC infusion.[2] Therefore, the dramatic loss of OZ1-treated cells in most patients may have arisen from any or all of these potential factors.

Although a past gene therapy trial using similar gammaretroviral vectors was successful in the treatment of 9 of the 10 SCID-X1 patients, four of the nine patients developed T-cell leukemia at 2.5–6.5 years after treatment because of gammaretroviral integration near proto-oncogenes.[7] Due to the apparent risks associated with gammaretroviral vectors, it will be imperative to monitor the patients for uncontrolled, retroviral-induced clonal expansion of OZ1 cells over the 15-year follow-up period. The scale of this trial and the diverse medical histories of the cohort will also provide a fascinating study for long-term follow-up of T-cell and viral dynamics. Finally, the researchers should explore the possibilities for OZ1 ineffectiveness, including viral evolution and retroviral silencing.

Although there are clear limitations of the OZ1 strategy, other promising gene therapy approaches await. Lentiviral vectors, which favor integration near active genes, do not appear to activate proto-oncogenes and thus may serve as a safer and more effective alternative to gammaretroviral vectors.[2] To circumvent the possibility of mutational escape from the virus, the inhibition of virus-dependent host genes, such as the T-cell chemokine receptor 5, can effectively block viral infection.[8–10] Combining multiple anti-HIV agents within a single-gene therapy vector might further reduce the effective dose of each component and the potential for viral escape.[2,8] Thus, although the pace remains incremental, the promise of gene therapy for HIV remains hopeful.

Conflict of interest

Dr Rossi's research is funded by the NIH. He is also a scientific advisor to Benitec, which has an interest in RNA interference-based gene therapy for HIV treatment. Dr Burnett declares no conflict of interest. ■

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