HIV eradication—from Berlin to Boston

To the Editor:

The requirement for lifelong combination antiretroviral therapy (cART) to treat HIV-1 infection creates a burden for both the affected individual and society and is a result of the long-term persistence of HIV-1 reservoirs in the body[1]. Consequently, there is great interest in the recent reports of four individuals who may have been cured of the virus after stopping cART, informally referred to as the 'Berlin patient', the 'Mississippi baby' and the two 'Boston patients'[2–4]. Additionally, at least one other baby (the 'Los Angeles baby') was recently reported as having undetectable HIV-1 reservoirs, although the baby is currently being maintained on cART[5]. Understanding the basis for their individual outcomes could inform future therapies aimed at suppressing or eradicating HIV-1 reservoirs and allowing drug-free management of the disease.

Timothy Henrich speaking about the two Boston HIV-positive patients at the 7th IAS Conference on HIV Pathogenesis, Treatment and Prevention in Kuala Lumpur, Malaysia. Source: ©International AIDS Society/Steve Forrest/Workers' Photos

In the first reported case of a cure, the HIV-positive Berlin patient received two hematopoietic (or blood) stem cell transplants during treatment for leukemia. Of note, the stem cell donor was both an appropriate tissue match and also homozygous for a 32-bp deletion in the chemokine CC-motif receptor 5 gene (*CCR5Δ32*). This mutation removes an essential HIV-1 entry co-receptor, and homozygotes have high levels of resistance to the virus. The transplantation was undertaken in the belief that these cells could provide the patient with a replacement, donor-derived immune system that was also HIV-resistant. To date, the patient has gone more than five years with no detectable HIV-1 in his body, despite cessation of all anti-HIV drug therapy since before the first transplantation[2,6]. However, the leukemia treatment also included an aggressive cytoablative conditioning regimen to destroy the cancer, and graft-versus-host disease (GVHD) developed, where donor-derived T cells can attack any residual leukemia, but in this case they could also have targeted any remaining HIV-infected cells in his body that formed the reservoir of the disease. This leads to the formal possibility that the HIV-resistant genotype of the donor was irrelevant, or played only a minor role, in the positive outcome.

In this regard, initial reports[3] of a lack of detectable HIV-1 in two previously HIV-positive Boston lymphoma patients, who also received cytoablative conditioning and stem cell transplantations, but not CCR5-negative cells, had suggested that it might be possible to achieve complete eradication of HIV-1 infection without the burdensome requirement to find a matched *CCR5Δ32* donor. Such an outcome would influence therapy for future transplant recipients, but could also suggest what might be required to achieve a cure in the much larger group of HIV-infected individuals who will never need to undergo such treatments. The recent news that the virus has returned in both Boston patients following cART withdrawal[7] is disappointing, but may still provide important information about the elements that contributed to success for the Berlin patient.

The cases of the Berlin patient and the two Boston patients have much in common. All three individuals underwent fully ablative conditioning during their cancer treatments, and received stem cell transplants from allogeneic (non-self) donors. Stated more specifically, the Berlin patient received an initial myeloablative allogeneic transplant followed by a second nonmyeloablative transplant from the same *CCR5Δ32* donor, whereas the Boston patients received initial myeloablative autologous (self) transplants followed several years later by nonmyeloablative allogeneic transplants. All three patients developed GVHD. Even so, a key difference is how their donor-derived immune systems could have been protected from HIV-1 during these procedures. Unlike the Berlin patient, the two Boston patients did not receive CCR5-negative cells, but were maintained on cART throughout the whole procedure, providing the possibility of chemically protecting their new, donor-derived cells.

It was initially reported that HIV-1 had become undetectable in both Boston patients as they progressed to full donor engraftment, and they exhibited waning anti-HIV immune responses[4]. To formally establish a cure, however, one requires a proof of absence that current sampling methods cannot deliver. In this regard, temporary withdrawal from cART is considered an experimental manipulation to test whether virological clearance has occurred because drug withdrawal typically results in rapid viral rebound as HIV-1 reemerges from its reservoirs. If such an analytical treatment interruption does not lead to rebound, it may be taken as evidence of viral suppression

and at least a functional cure, although the duration of remission needed before proclaiming a permanent or sterilizing cure is unknown. Indeed, in the absence of such information, a more suitable description of the Berlin patient's ongoing status might be 'long-term remission' rather than 'cure'.

Following analytical treatment interruption, it is now apparent that neither Boston patient had achieved complete viral eradication during their cancer treatments, although their HIV-1 rebounded more slowly than is typical. In hindsight, this outcome may not be all that surprising, given the variety of outcomes that follow allogeneic transplantation in patients with malignant hematologic diseases. Here, a graft-versus-leukemia effect is often an essential component to effectively eradicate or control any remaining cancer, but understanding the factors that can influence the outcome has proven challenging[8]. Similarly, in HIV-positive transplant recipients, the effectiveness of a donor graft-versus-HIV-reservoir effect could also be expected to be variable. In addition, although infected memory $CD4^+$ T cells have been identified as an important reservoir of the virus that persists during cART[1], other viral reservoirs, including non-T cells, may not be destroyed by an allogeneic response.

Although their HIV-1 was not eradicated, a question that arises in the case of the Boston patients is whether the treatment they received could nonetheless have reduced their HIV-1 reservoirs and provided clinical benefit, even without reaching the status of a cure? Smaller HIV-1 reservoirs are associated with reduced pathologic sequelae, such as inflammation, that occur even with effective cART control of viremia[9]. However, this potential beneficial effect would continue only under cover of cART; once the Boston patients were removed from this pharmacological safety net, any reactivation of HIV-1 from remnants of their original reservoirs would readily infect the donor-derived cells, effectively mimicking a primary HIV-1 infection, and potentially seeding more extensive viral reservoirs. Such a scenario means that the risks and benefits for analytical treatment interruption in such patients need to be carefully considered and discussed.

The failure of the Boston patients to maintain HIV-1 suppression off cART reveals that even the intense cytoablative conditioning and GVHD that they experienced did not fully eradicate all HIV-1 reservoirs, and that transplantation of HIV-resistant cells from the *CCR5Δ32* donor was likely an essential component in the salutary outcome for the Berlin patient.

With this in mind, efforts are underway to mimic this aspect of the therapy by engineering autologous T cells or hematopoietic stem cells to be HIV-resistant, and enormous progress has been made in developing techniques to render cells resistant to HIV-1 (ref.10). These include methods that specifically create CCR5-negative cells, through the use of either RNA interference or zinc-finger nuclease (ZFN) technologies[11,12].

Last month, Tebas *et al.*[13] reported results from the first small trial evaluating CCR5 gene disruption by ZFNs, in which 12 HIV-positive patients underwent adoptive transfer of autologous CD4 T cells that had been treated *ex vivo* with an adenoviral vector encoding ZFNs targeting CCR5. Some intriguing indications of efficacy were observed. For example, although viremia recurred in all patients for whom antiretroviral therapy was transiently interrupted and was accompanied by an expected decrease in CD4 T-cell levels, the decline in the levels of CCR5-modified cells was significantly less pronounced than for the total T-cell population. In addition, one study participant, later found to be heterozygous for the *CCR5Δ32* allele, managed to fully control his viremia during the intervention. This observation fits with the requirement for biallelic CCR5 disruption to achieve HIV resistance, which would be easier to achieve in this genetic background. Current methods to deliver ZFNs to T cells and hematopoietic stem cells result in 33–40% bialleleic disruption in the edited cells[14,15], but more efficient ZFN delivery or expression may be required to increase this frequency for individuals with two functional copies of CCR5.

Ultimately, it is not yet known whether HIV-resistant cells will be effective in the autologous setting, without the accompanying chemoablation or GVHD that could reduce infected cell reservoirs. However, it should also be possible to engineer HIV-resistant stem cells for patients requiring allogeneic transplantation, to more fully mimic the Berlin patient protocol, and thereby evaluate whether a graft-versus-reservoir effect is necessary for full eradication. Meanwhile, the differing outcomes for both the Berlin and Boston patients, although highlighting the pernicious nature of the HIV-1 reservoirs and the formidable challenges that HIV-1 eradication strategies face, also provide impetus for the continued development of therapeutic interventions that include the introduction of HIV-resistant cells.

ACKNOWLEDGMENTS

The authors acknowledge support from the National Institutes of Health grants U19 AI096111, P01 HL73104 and R01 AI080326, and the California Institute for Regenerative Medicine grant DR1-01490.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Finzi, D. *et al. Nat. Med.* **5**, 512–517 (1999).
2. Allers, K. *et al. Blood* **117**, 2791–2799 (2011).
3. Persaud, D. *et al. N. Engl. J. Med.* **369**, 1828–1835 (2013).
4. Henrich, T.J. *et al. J. Infect. Dis.* **207**, 1694–1702 (2013).
5. Persaud, D. *et al.* 21st Conference on Retroviruses and Opportunistic Infections. Abstract 75LB (March 3–6, 2014).
6. Yukl, S.A. *et al. PLoS Pathog.* **9**, e1003347 (2013).
7. Hayden, E.C. *Nature News* doi:10.1038/nature.2013.14324 (6 December 2013).
8. Warren, E.H. & Deeg, H.J. *Tissue Antigens* **81**, 183–193 (2013).
9. Klatt, N.R. *et al. Immunol. Rev.* **254**, 326–342 (2013).
10. Kiem, H.P. *et al. Cell Stem Cell* **10**, 137–147 (2012).
11. Burke, B.P. *et al Viruses* **6**, 54 -68 (2014).
12. Cannon, P. & June, C. *Curr. Opin. HIV AIDS* **6**, 74 -79 (2011).
13. Tebas, P. *et al. N. Engl. J. Med.* **310**, 901 -910 (2014).
14. Perez, E.E. *et al. Nat. Biotechnol.* **26**, 808-816 (2008).
15. Li, L. *et al. Mol. Ther.* **21**, 1259 -1269 (2013).

The time is ripe for an ethics of entrepreneurship

To the Editor:

Entrepreneurship has been hailed as an important engine for economic growth, productivity, innovation and employment. During the recent economic crisis, entrepreneurship has been cited as the key to reducing unemployment, unleashing the potential of the social economy, and delivering on the promise of new biomedical and green technologies (http://www.oecd.org/cfe/leed/leedprojectsyoucanjoinin2011-2012.htm). In higher education, entrepreneurship programs have become the fastest-growing field of study on campus and have resulted in the emergence