Regenerating blood: towards engineering HIV-1-resistant hematopoietic stem cells

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“The good news is that since we have the one proof of concept from the Berlin patient, AIDS advocates have come around to the fact that this is now possible, it’s not a pipe dream, it’s been done once. Let’s do it again, in a different way.”

– Jeffrey Laurence, professor of medicine at the Cornell University (NY, USA) [1].

“The importance of our findings is that we have evidence now that we can protect uninfected cells from becoming infected when they’re transplanted into an HIV-infected patient – a form of prep at the cellular level, if you will. And these data give further encouragement to the field and provide another piece of the puzzle as we continue our work towards a cure that will be generalizable and applicable to HIV patients worldwide.”

– Daniel Kuritzkes, professor of medicine, Brigham and Women’s Hospital in Boston (MA, USA) [2].

It has been now three decades since HIV-1 was first recognized and nearly 20 years since combined antiretroviral therapy (HAART) improved the outcome of the infection effectively. However, although this success story has helped to transform HIV-1 infection into a more chronic disease, there is still no vaccine or curative treatment available. Additionally, medicines to treat HIV-1, particularly the newest and most tolerable drugs, are expensive and only available in high-income countries, whereas the peak of infected people live in low- or middle-income countries.

Therefore, there is a rising demand in finding feasible and inexpensive strategies to cure the infection. In this context, cure means to get rid of the antiretroviral medication and

concurrently control virus replication and disease progression (so-called functional cure). It is still unclear and discussed controversially, whether a complete eradication of the virus is achievable (sterilizing cure) [3]. An up-to-date overview on current cure strategies is available at [4].

The cure debate became thick and fast after the intriguing reports of cases like the ‘Boston patients’ or the ‘Mississippi baby’ in which individuals displayed a period of viral control without HAART, but at least rebounded after the antiretroviral medication was discontinued over a critical point of time [5,6]. The only and most compelling evidence of a medical intervention that cured HIV-1 infection has been a successful hematopoietic stem cell transplant in an adult-infected patient (the ‘Berlin patient’), transferring donor-derived cells from an individual homozygous for the *CCR5-Δ32* deletion, which confers resistance against HIV-1 transmission. The lack of rebounding virus after 6 years without therapy, the failure to isolate infectious virus and the fading HIV-specific immune responses indicate that the patient has been effectively cured [7,8].

The key of the success of the ‘Berlin patient’ was the observation that individuals with a decreased or missing CCR5 receptor, which forms together with CD4 the main entrance complex for HIV-1, are less susceptible or completely resistant against HIV-1 transmission [9]. The ‘Berlin patient’ is to be considered as a proof-of-principal and constitutes as evidence that any – in this case ‘natural’ – gene therapy might work to eradicate the virus. The general idea of an HIV-1

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gene therapy is probably as old as the detection of the virus itself but was most emphasized by Nobel prize laureate David Baltimore in the late 1980s. After that a bunch of different strategies aiming certainly more than 30 different targets and using all kinds of available vectors have been developed and tested and found ineffective to reduce the viral burden or to control replication.

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HIV-1 gene therapy is generally grouped into three classes, based on the targeted phase of viral life cycle [10]. After the success of the ‘Berlin patient’ experiment, gene therapy approaches focus now on the class I target: the HIV-1-entry mechanism and especially the CCR5 chemokine coreceptor. Most advanced are two approaches to knock down the CCR5 receptor: the ‘classical’ gene therapy strategy with lentiviral vectors and the new developed technique of zinc finger nucleases (ZFNs) using a simple adenovirus.

During the last decade, Sangamo a biotech company located in Richmond (CA, USA), developed a specific ZFN that induces a double-strand break in the *CCR5* gene, leading to a nonfunctional receptor and thereby mimicking the natural occurring *CCR5*-Δ32 deletion. However, a complete suppression of all CCR5 expression on the cell surface is not feasible with any currently used gene therapy technique. In several Phase I trials the dynamics and the outcome of ZFN-manipulated cells were tested. HIV-1-infected patients donate their autologous T lymphocytes, which were expanded and transduced by the adenovirus vector and then re-infused. All patients had an increment of in terms of HIV infection critical CD4⁺ cell count indicating an improvement of the immune state. Furthermore, these transduced T cells expanded and reseeded the immunological important sides like the gut as tested in several biopsies. In a second experimental setting, the antiretroviral medication was discontinued after re-infusion of ZFN-manipulated cells to increase the selective pressure and to enhance the expansion of artificial HIV-resistant cells. As expected, all patients had a quick and reasonable rebound of the virus, but interestingly, in those patients found heterozygous for the CCR5-Δ32 deletion some displayed signs of a real virus control [11].

This could imply that a complete suppression of the CCR5 receptor is not mandatory to confer resistance

or virus control and that there might be a critical receptor density to maintain replication and spread of the virus. Follow-up trials of this approach, for example, using immune-suppression during cell transfer, are in progress.

The second promising approach is featured by Calimmune Inc. (LA, USA), which uses a sh-RNA lentiviral construct to downregulate CCR5 in combination with a small peptide linker C46 (together called Cal-1) that interacts with the fusion of the virus and the target cell surface [12]. There some advantages of this approach: first, the sh-RNA technique is thought to be superior to suppress CCR5 more efficient than other approaches including ZFN, and second, the combination of two independent mechanism might be more sufficient to achieve a higher grade of entry barrier, and third, other peptide linkers like C34 that could be combined with this technique work even with other receptors despite CCR5 [13]. This is an important point because HIV-1 is able to escape CCR5 withdraw by a quite simple mutation of his V3 loop and thereby achieves the ability to use other chemokine receptors like CXCR4 to invade the cells.

“...observations of natural occurring CCR5 mutations and the first results of suppressing CCR5 in HIV-1-infected people are suggestive that a complete knockout is not required to disrupt viral replication and spread.”

The first patient in the Calimmune group was treated in a Phase I study (NCT01734850) in late June 2013 testing the safety and feasibility of the Cal-1 approach. This study will only include patients with a history of previous antiretroviral therapy who currently discontinued their medication and have a stable immunological situation. Participants receiving the Cal-1 gene transfer will be merged into three arms: two arms of them receive different amounts of busulfan as a preconditioning regimen. The additional use of immunosuppressive medication is thought to help the transfused manipulated cells to expand and overcome the old immune system with the latently infected reservoirs. The company announced that first data for 2015 to be published [14].

Outlook

HIV-1 has revealed his Achilles’ heel by preferentially and undeviatingly using CCR5 as entry receptor which is, in opposite to CD4, dispensable for human beings and therefore displays the major target for suppressive or knockout strategies. Furthermore, observations of natural occurring CCR5 mutations and the first results of suppressing CCR5 in HIV-1-infected

people are suggestive that a complete knockout is not required to disrupt viral replication and spread. Current work on HIV-1 gene therapy has opened the promise of a single shoot treatment that might replace, or at least amend, current antiretroviral lifelong treatment. However, current trials are in very early state of development and potential risks of manipulation of autologous cells or even stem cells are not foreseeable at the this time.

Financial & competing interests disclosure

G Hütter is a clinical advisor for Calimmune Inc. He has no financial involvement with Calimmune Inc. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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References

- 1 The AIDS beacon™. www.aidsbeacon.com
- 2 Democracy NOW! www.democracynow.org
- 3 Lewin SR, Deeks SG, Barre-Sinoussi F. Towards a cure for HIV – are we making progress? *Lancet* 384(9939), 209–211 (2014).
- 4 Treatment Action Group. www.treatmentactiongroup.org/cure/trials
- 5 Henrich TJ, Hanhauser E, Marty FM *et al.* Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. *Ann. Intern. Med.* doi:10.7326/M14-1027 (2014) (Epub ahead of print).
- 6 Persaud D, Gay H, Ziemniak C *et al.* Absence of detectable HIV-1 viremia after treatment cessation in an infant. *N. Engl. J. Med.* 369(19), 1828–1835 (2013).
- 7 Hütter G, Nowak D, Mossner M *et al.* Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N. Engl. J. Med.* 360(7), 692–698 (2009).
- 8 Yukl SA, Boritz E, Busch M *et al.* Challenges in detecting HIV persistence during potentially curative interventions: a study of the Berlin patient. *PLoS Pathog.* 9(5), e1003347 (2013).
- 9 Catano G, Chykarenko ZA, Mangano A *et al.* Concordance of *CCR5* genotypes that influence cell-mediated immunity and HIV-1 disease progression rates. *J. Infect. Dis.* 203(2), 263–272 (2011).
- 10 von Laer D, Baum C, Protzer U. Antiviral gene therapy. *Handb. Exp. Pharmacol.* 189, 265–297 (2009).
- 11 Tebas P, Stein D, Tang WW *et al.* Gene editing of *CCR5* in autologous CD4 T cells of persons infected with HIV. *N. Engl. J. Med.* 370(10), 901–910 (2014).
- 12 Savkovic B, Nichols J, Birkett D *et al.* A quantitative comparison of anti-HIV gene therapy delivered to hematopoietic stem cells versus CD4⁺ T cells. *PLoS Comput. Biol.* 10(6), e1003681 (2014).
- 13 Augusto MT, Hollmann A, Castanho MA *et al.* Improvement of HIV fusion inhibitor C34 efficacy by membrane anchoring and enhanced exposure. *J. Antimicrob. Chemother.* 69(5), 1286–1297 (2014).
- 14 Burke BP, Boyd MP, Impey H *et al.* *CCR5* as a natural and modulated target for inhibition of HIV. *Viruses* 6(1), 54–68 (2014).