Stem cell-based gene therapy in HIV treatment

Fernando Pimentel
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Introduction

In 2007, an HIV infected patient, Timothy Ray Brown, underwent allogeneic hematopoietic stem cell transplantation (HSCT) as a treatment for acute myeloid leukemia (AML). Gero Hütter, an oncologist and hematologist, who performed the procedure, posed the radical question: What if a stem cell transplant could eliminate both the leukemia and HIV? Hütter knew from reading the literature that *CCR5* is a major co-receptor, found in T-cell subsets, and is utilized by HIV-I to gain entry into these cells; these T-cell subsets are depleted during HIV-infection (deRoda Husman et al, 1999). Furthermore, it has been established that a genetic 32-bp deletion in *CCR5* (Δ32), which is relatively common in Western European populations, confers resistance to HIV-infection and AIDS in Δ32 homozygotes (Samson et al, 1996).

This led Dr. Hütter to search for a HSCT donor-match who was also homozygous for the *CCR5* mutation with the goal of eradicating AML with a drug regiment and replacing Brown's immune system with an entirely new blood-forming system containing the *CCR5* mutation. After a relapse of AML following the first HSCT attempt, Brown underwent a second transplant eleven months later with the same donor. This time the drug regiment for immune system ablation was coupled with whole-body irradiation. The second HSC transplant was a success, and to this day Brown has achieved disease remission and despite discontinuing antiretroviral drugs (ARVs) more than 4 years ago, Brown has an undetectable viral load and no latently infected cells can be found anywhere in his body (Hutter et al, 2009)

This had led to investigations that seek to sidestep the HSCT and focus on gene therapy involving the *CCR5* gene. Efforts led by Paula Cannon et al, have utilized adenoviruses containing zinc-finger nucleases (ZFNs), which clip out a specific small sequence of DNA in the *CCR5* gene and render cells incapable of producing a functional version of the receptor. This was translated into a procedure in which ZFN-treated human CD34+ hematopoietic stem/progenitor cells (HSPCs) were transplanted into NOD/SCID.IL2rγ^{null} mice. The results showed the mice transplanted with ZFN-modified HSPCs underwent rapid selection for *CCR5* -/- cells, and were associated with significantly lower HIV-I levels (Cannon et al, 2010).

This demonstration that a minority of CCR5 -/- HSPCs yield cells competent to engraft, support hematopoiesis, and maintain anti-viral benefit - has opened up treatment to many more HIV-infected individuals. Sangamo BioSciences, Inc has recently taken this approach and applied it to HIV-infected patients. In their most recently published Phase 2 clinical trials, infusion of zinc finger nuclease *CCR5* modified autologous CD4 T Cells (SB-728-T) has been shown to increase CD4 counts and decrease HIV proviral load in HIV-infected subjects when ARVs are withdrawn during treatment interruption (CROI Abstract # 155, 2012). Collectively, these demonstrations validate the use of SB-728-T and other comparable genetically modified early cytokines as an effective strategy in finding a “functional cure” for HIV.

Cell-surface receptor CCR5

David Baltimore first coined the term “intracellular immunization” to describe the process of integrating HIV resistant genes into HSCs to allow persistent repopulation of the host with progeny cells that would be impervious to HIV infection (Baltimore, 1988). Ever since then, there has been an increasing effort to devise techniques and vectors for genetic modification of human HSCs in preparation for transplantation. More recently, this effort has been revitalized with the identification of *CCR5*, the cell-surface receptor, which has opened up a new avenue for the targeting of HIV.

HIV-I entry into target cells requires the binding of the viral gp120 Env protein to the CD4 receptor and a chemokine co-receptor (Wu et al, 2010). To gain entry, HIV-1 must utilize the *CCR5* chemokine receptor (R5 variants) present on many immune cells

(Moore et al, 2004). There is also another chemokine receptor which serves the same function in granting entry to HIV – CXCR4 (X4 variants). As HIV infection progresses, HIV evolves and often expands its co-receptor preference to include CXCR4; however, the vast majority of transmitted viruses use CCR5 (Moore et al, 2004).

A small proportion of individuals of Western European descent have been found to contain a genetic 32-bp deletion in *CCR5* (Δ32), which confers resistance to HIV-I infection. Moreover, individuals homozygous for the mutation, showing absence of *CCR5*, have demonstrated very low risk of HIV-infection. Individuals heterozygous for the *CCR5* (Δ32) deletion demonstrate delayed disease progression after they acquire HIV (Moore et al, 2004).

While there is some evidence that suggests *CCR5* (Δ32) may be associated with an increased risk of some uncommon infections, there is also evidence that suggests the *CCR5* deletion is associated with reduced risk of certain inflammatory diseases. Furthermore, there does not appear to be any significant difference in life expectancy. For these reasons, the discovery of *CCR5* (Δ32) has elucidated a strategy for HIV-protection that may be well tolerated in patients (Glass et al, 2006).

Berlin Patient

One of the milestones for what some call a “functional cure” of HIV is the HSCT with homozygous *CCR5* (Δ32) HLA-matched HSC cells of Timothy Ray Brown. Viral loads in HIV-infected individuals can remain undetectable in peripheral blood with the use of ARVs, however, Brown’s case was exception in that after HSCT no latently infected cells (memory HIV-infected cells) could be found in any of Brown’s tissues. However, while this example is as close to a “cure” that anyone has seen, it is important to emphasize that the road to this “cure” was an unlikely one and an arduous one that will not be available to even a small minority of HIV-infected patients.

The Berlin patient had to undergo two transplantations due to a subsequent relapse of AML after the first attempt. Brown underwent a fully ablative conditioning regiment with a complex and potentially lethal regiment that consisted of fludarabine (Fludara), Ara-C, amsacrine (Amerkin, Amsidyl, Amsidine), cyclosporine, mycophenolate motefil

(CellCept), antithymocyte globulin and 4 Gy of total body irradiation. Furthermore, his HSCT resulted in the onset of graft-versus-host-disease (GVHD), which will require a long-term immunosuppressive regiment (Deeks et al, 2011).

For these reasons, HSCT is hardly an effective treatment of HIV compared to the current use of ARVs. HSCT for the sole treatment of HIV infection is far too risky to justify the potential benefit of HIV management, which is currently managed safely and effectively with ARVs. HSCT with homozygous *CCR5* (Δ32) was performed because it was necessary to treat Brown's AML. Nonetheless, the success of this case has justified efforts aimed at developing *CCR5* (Δ32) targeted stem cell therapy.

Zing-finger nucleases

These observations of *CCR5* targeted gene therapy in HIV infected individuals have led to various investigations aimed at blocking *CCR5* gene expression. Some of these methods for *CCR5* disruption include the use of ribozymes, siRNA and intrabodies. These three approaches have been successful at blocking expression in both mature T cells and CD34+ HSPCs, and they do not appear to have any adverse effects on hematopoiesis (Swan et al, 2006). While these methods have been successful at achieving short-term disruption of *CCR5*, engineered zinc-finger nucleases (ZFN) targeted to *CCR5* offer a permanent gene disruption over multi-lineage progeny.

ZFNs comprise a series of linked zinc fingers engineered to bind specific DNA sequences and an endonuclease domain (Urnov et al, 2005). Concerted binding of two juxtaposed ZFNs on DNA is subsequently followed by dimerization of the endonuclease domains, which results in a double-stranded break at the specific DNA sequence. These double-stranded breaks initiate cellular repair pathways, including the mutagenic nonhomologous end-joining pathway, which causes additions and deletions of nucleotides at the break site. Consequently, this creates gene disruption that is passed to daughter cells in the absence of persistent transgene expression (Sonoda et al, 2006).

These zinc finger nucleases can be introduced *ex vivo* using methods which include integrase-defective lentiviral vectors, adenoviral vectors and plasmid nucleofection. ZFN-mediated disruption of *CCR5* in CD34+ HSPCs via plasmid nucleofection did not

show any adverse effects on cell viability compared to mock nucleofected controls. While there is evidence of toxicity compared to untreated cells, these adverse effects on cell viability are offset by the high levels of *CCR5* disruption as well as the speed and simplicity of the procedure. Furthermore, while less toxic methods of ZFN disruption have recently been designed, such as those by Sangamo BioSciences, Inc, this aforementioned method was essential in transferring this method into HIV-infected animal studies (Cannon et al, 2010).

Measuring engraftment and *CCR5* -/- selection of CCR5-targeted ZFN human CD34+ T cell in mice

Having established the ability of *CCR5*-targeted ZFN treatment to produce viable human CD34+ T cells with gene disruption, the next step was to evaluate these modified HSPCs' durable anti-viral effects and persistent gene disruption *in vivo*. These evaluations were performed in nonobese diabetic/severe combined immunodeficient/interleukin2rγ^{null} mice (NSG) mice, which support both human hematopoiesis and HIV-I infection (Kumar et al, 2008).

NSG mice were engrafted with ZFN-treated human CD34+ HSPCs that had received low-dose (150cGy) radiation. The human cells engrafted efficiently and rapidly, and the animals showed no obvious toxicity or ill health. 12 weeks after transplantation, engraftment analysis showed high levels of human cells in both peripheral blood and tissues, ranging from 5-15% of the intestine, >50% of blood, spleen and bone marrow, and >90% of the thymus. Furthermore, the profile of human cells in mice receiving ZFN–treated CD34+ HSPC was indistinguishable compared to mice receiving unmodified cells, with respect to percentage of cells in different tissues and frequency of different subsets, which suggests ZFN-modified CD34+ HSPCs are functionally normal (Cannon et al 2010).

Engrafted animals at 12 weeks post-transplantation of either unmodified or ZFN-treated CD34+ HSPCs were infused with CCR5-tropic virus HIV-1BAL. This strain of HIV-1 has been shown to cause robust infection and results in significant CD4+ T-cell depletion in humanized mouse models (Berges et al, 2006). FACS analysis of the spleen and intestine of ZFN-treated infected animals at 12 weeks following HIV-1

infusion showed undetectable CCR5 gene expression. In contrast, ZFN-treated uninfected animals showed ~25% of CD4+ cells were CCR5 +. These results confirm that HIV-I infection rapidly selects for CCR5 -/- T cells, which confers protection of CD4+ lymphocytes on ZFN-treated mice (Cannon et al, 2010).

Measuring the anti-viral effect of *CCR5*-targeted ZFN human CD34+ T cell in mice

To investigate the viral presence of HIV-I in the peripheral blood and tissues of animals, quantitative PCR analysis of HIV-RNA levels were measured. Peripheral blood analysis of infected mice showed peak viremia occurred 6 weeks post infection in mice receiving either ZFN-treated or untreated HSPCs. By 8 weeks post infection, HIV-I RNA levels continued to drop in both groups of mice, but these levels were statistically lower in mice with ZFN-treated HSPCs ($p=0.001$). The decrease in HIV-RNA levels in untreated mice was expected and attributed to the loss of CD4+ T cells as the infection progressed. In contrast, ZFN-treated mice showed a rebound in repopulation of CD4+ T cells 2 weeks post infection and recovered to normal levels by 4 weeks following infection (Cannon et al, 2010).

HIV-I levels were also measured in intestinal samples of ZFN-treated and untreated HIV-infected mice. Intestinal samples collected 8 and 9 weeks post infection showed that viral levels were 4 orders of magnitude lower in ZFN-treated mice compared to untreated mice. At 12 weeks following infection, HIV-RNA levels were undetectable by quantitative PCR in ZFN-treated mice. These significant drops in viral levels did not have any adverse effects on healthy human CD4 T cells; normal maintenance levels of healthy T lymphocytes were present in the intestines and other tissues. These results suggest that ZFN-treated mice have protective-viral effects, have strong selective pressure for CCR5 -/- cells, and maintain normal levels of healthy T lymphocytes in tissues and peripheral blood. (Cannon et al, 2010).

Sangamo's ZFP Therapeutic®

The research and development of ZFNs, such as the evaluations discussed in Cannon

et al 2010, has been a collaborative effort with the scientific team at Sangamo BioSciences, Inc. They have engineered a class of DNA-binding proteins, zinc finger DNA-binding proteins (ZFPs), which are delivered via a viral vector (adenoviral vector) into T-cells. These ZFPs permanently knockout the expression of the CCR5 protein in T-cells, and yield an autologous CD4 T-cell product called SB-728-T.

On the basis of the aforementioned laboratory results, there is the potential that SB-728-T may work in HIV-infected humans and improve their immune system by allowing their CD4 T-cells to survive longer. This has led to Sangamo BioSciences' pursuit of two clinical trials with the purpose of determining whether SB-728-Ts are safe in humans and to determine their effects on HIV.

Clinical Trial: Autologous T-Cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases SB-728 for HIV (Zinc-Finger)

This clinical trial (NCT00842634) included 3 cohorts of patients to include a total of 21 HIV-infected subjects. Cohort 1 included patients who failed two or more HAART regimens; cohort 2 included patients doing well on stable ARVs; and cohort 3 included patients with an undetectable viral load on HAART who have exhibited suboptimal CD4+ T cell gains during long term ARVs (NCT00842634).

The trial began with a physical exam, medical history, and blood draws for clinical labs and research. This was followed by two apheresis procedures to collect T cells from each respective patient. The second apheresis occurred at approximately 3 weeks and was followed by a rectal biopsy. Then each patient went through a physical exam, and blood draw for clinical labs and research approximately 2 weeks following the second apheresis procedure. 2 weeks after this clinical visit, patients received a single infusion of 5-10 billion ZFN Modified CD4+ T Cells. Additionally, cohort 2 patients, those doing well on stable ARVs, were instructed to stop taking HAART medication for up to 12 weeks (4 weeks after infusion to 16 weeks after infusion). In cohort 2, ARVs were restarted 20 weeks after ZFN modified T cell Infusion. For all three cohorts, follow up clinic visits occurred 48, 72 hours; 1,2,3,6 weeks; 2,3,6, 9 months after ZFN infusion (NCT00842634).

At the 19th Conference on Retroviruses and Opportunistic Infections (CROI), held in

Seattle from March 5 to March 8, 2012, Sangamo BioSciences, Inc. announced its most recent data from its ongoing Phase 2 Trials.

One month after SB-728-T treatment, six subjects underwent a 12-week treatment interruption (TI) of HAART. During TI, HIV-RNA levels initially increased as expected in all 6 subjects. Subsequent reduction in viral load of 0.8 to > 2.0-log from peak viremia was observed in 3 of the 6 subjects – these subjects also had the highest estimated circulating levels of biallelic modification of their *CCR5* gene. One of these subjects (subject 205) had an undetectable viral load such that the subject was avimeric at the end of TI. Subject 205 entered the clinical trial with one copy of the natural *CCR5* Δ32 mutation, thereby this subject's percentage of biallelically-disrupted *CCR5* genes (i.e. modification of both copies) was twice that of the 20 other subjects who entered the study with wild-type *CCR5* (CROI Abstract # 155, 2012).

This most recent data shows that viral load suppression correlated significantly ($p<0.05$) with levels of circulating CD4+ T-cells with biallelic modification. Furthermore, the level of circulating viral DNA was elevated in only one subject; whose SB-728-T cell engraftment was also the lowest of the group.

With regards to engraftment, there was a range of persistence from 90 to 738 days, but generally SB-728-T persists in the peripheral blood for over a year. SB-728-T was also found to traffic to the gut mucosa, which is an important reservoir of active HIV infection. Moreover, SB-728-T treatment resulted in elevated levels of certain T-cell cytokines (IL-2, IL-7 and IL-15) in the peripheral blood immediately following SB-728-T infusion, which may have a role in the robust expansion of CD4+ T-cells (CROI, Abstract # 155, 2012).

More importantly, SB-728-T treatment continues to be safe and appears well tolerated with only mild, but reversible symptoms, which are typical of infusion reactions (CROI Abstract # 155, 2012). This data presented at CROI 2012 continues to validate the strategy of gene therapy as a safe and potentially "functional cure" for HIV. Completion of this ongoing Phase 2 clinical trial will further elucidate the motivations behind this effort.

Questions and concerns

While clinical trials of stem cell-based gene therapy are steadily advancing, there are still many unanswered questions and a need to address these practical concerns before this treatment becomes a solid reality in the clinic. One of the loudest concerns has been regarding the toxicity of ongoing viral replication, and whether patients and their providers will be willing to allow HIV to replicate at high levels in the absence of ARVs so that *CCR5* -/- CD4+ T cell selection can occur. Evidence shows that HIV replication causes significant and perhaps irreversible harm to the cardiovascular, renal, hepatic, and neurological systems (Phillips et al, 2008). Thus, the design of the clinical trial NCT00842634, such that subjects undergo treatment interruption, is an ongoing concern and is assumed to carry some risk.

A second concern is the uncertainty of whether *CCR5*-deficient cells may select for the outgrowth of a resistant HIV-1 population. This concern is especially daunting given that HIV X4 variants (i.e. viruses which use the *CXCR4* chemokine receptor to gain entry) are much more virulent than R5 variants (i.e. viruses which use the *CCR5* chemokine receptor) (Moore et al, 2004). Furthermore, the history of HIV therapeutics has demonstrated that if HIV replicates in the presence of a selective pressure it will with time selectively outgrow and survive.

Finally, a widely held concern has been the use of an ablative therapy as a necessary regiment for stem cell engraftment. Patients in need of aggressive intervention, like gene-based HSC therapy, would include those who typically have dual-tropic viral infection. Furthermore, it is uncertain whether these patients with advanced disease (i.e. damaged hematopoietic microenvironments in the bone marrow, thymus and lymph nodes) would be able to tolerate long-term or even-short term toxicity from an aggressive ablative treatment.

Conclusion

The success of the “Berlin Patient” has opened up a new avenue of treatment, whereby HSC—based gene therapy may serve as an additional tool in our reservoir of HIV therapeutics. For the past 15 years, ARVs have proven to be an effective strategy in stabilizing and stunting HIV replication. However, despite their potency, there are inherent limitations with ARVs in that they are not curative and patients must adhere to

these potentially toxic drugs for life as long as HIV persists indefinitely. The success of Timothy Ray Brown's *CCR5* -/- HSCT and the efforts led by Sangamo BioSciences, Inc to push these applications into clinical trials, has paved the road towards finding a "functional cure" for HIV infection. Lastly, even if *CCR5*-gene disruption is not the ultimate solution to this "functional cure," these clinical studies will add valuable lessons to our understanding of HIV pathogenesis and human immunology.

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