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Fighting HIV with stem cell therapy: one step closer to human trials?

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"It seems that the transplantation of HIV-resistant hematopoietic stem cells was able to generate a new immune system capable of controlling HIV replication."

In spite of the progress made with combination antiretroviral therapy (ART) over the past 15 years, HIV remains a major public health problem with no cure or preventative vaccine. WHO estimates the number of people living with HIV worldwide in 2010 at 34 million [101], with approximately 2.7 million newly infected individuals in 2010 [102]. Total AIDS deaths in 2010 were estimated to be 1.8 million [102]. Infected patients need to take ART under high compliance as otherwise the virus will rebound. Although it has saved many lives, ART has serious drawbacks. Long-term effects are not well known, particularly in aging HIV-infected populations. Additionally, resistant viral mutants can arise and the cost of expensive life-long drug therapy is a huge burden, both on the society and the individual. Clearly, alternative therapies are needed and the quest for a cure should be at the top of our research goals.

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In 1988, in an article published in *Nature*, David Baltimore coined the phrase 'intracellular immunization,' exploring the possibilities of gene therapy to develop a treatment for HIV based on 'anti-HIV genes' inserted into HIV target cells [1]. At that time, gene therapy was a new field and techniques were inefficient. However, members of our research group have been participating in the development of gene therapies, particularly stem cell gene therapy for HIV, from the very onset.

Hematopoietic stem cells (HSCs) are the source for all HIV target cells, including T cells, macrophages, dendritic cells and brain microglia. HIV permanently integrates into these cells and also forms 'reservoirs' of infected cells that cannot be eliminated. Therefore, we developed a hypothesis that states: if anti-HIV genes, which can protect HIV target cells from infection and replication, are inserted into HSCs, all progeny arising from these cells would resist infection. If all or most of the HSCs of an HIV-infected individual could be replaced with gene-modified stem cells, an HIV-resistant immune system could be generated, which would control HIV replication without the need of ART. T cells expressing anti-HIV genes would have a selective survival advantage over unprotected T cells and will increase in numbers in the face of a viral load, adding protection when needed. Such an HIV-resistant immune system may also control latent viral reservoirs over the long run, possibly leading to a postulated functional cure.

In the early 1990s, before the onset of combination drug therapy, the development of stem cell gene therapy for HIV rapidly progressed, which included single anti-HIV genes such as antisense RNAs, RNA decoys, ribozymes and transdominant negative proteins [2,3].

Keywords: anti-HIV genes • gene therapy • HIV cure • stem cells

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Potent molecules were translated into clinically applicable products and applied in Phase I clinical trials in the USA. Some centers transferred these genes into peripheral blood T cells, while others, such as our group, chose autologous HSCs as targets. Vectors based on the Moloney murine leukemia virus (a retroviral vector) were utilized in most studies, although some trials also used nonviral gene transfer methods [4].

In these clinical trials, bone marrow stem cells were mobilized from adult patients or bone marrow aspirates were taken from pediatric patients, and $CD34^+$ cells were purified and transduced with retroviral vectors. Transduction efficiency was measured and varied from 10 to 40%. Cell marking in the peripheral blood of patients, unfortunately, was low, often below 1% [5]. One pioneering clinical trial at that time was conducted in patients with HIV lymphoma who received high-dose chemotherapy with complete marrow ablation and an autologous bone marrow transplant to cure the lymphoma, while half of their bone marrow stem cells were also transduced with anti-HIV genes. Initially, patients in this trial displayed good gene marking in the periphery; however, this declined over time. ART had become standard of care and regulatory agencies required all patients enrolled to continue taking ART. The drug therapy, however, suppressed viral load, and a selective survival advantage of anti-HIV gene expressing cells in the setting of HIV replication could not be demonstrated.

There was a single case in a pediatric clinical trial, however, that demonstrated anecdotal evidence of a selective survival advantage. This patient received transduced bone marrow $CD34^+$ cells without marrow conditioning and displayed low peripheral blood gene marking, which eventually disappeared over 6 months of continuing ART. After approximately 12 months, the patient stopped taking ART due to side effects, and gene marking of anti-HIV gene transduced cells appeared again in the peripheral blood, rising significantly with an increase in viral load. The patient was convinced to begin drug therapy again, upon which gene marking in the peripheral blood and the viral load decreased [6].

> "Our translational studies have progressed to the stage where we have designed a clinical protocol for a new HIV stem cell gene therapy clinical trial..."

Researchers evaluated the outcomes of these clinical trials and concluded that retroviral vectors were not adequate gene transfer vehicles and also posed safety risks due to their inherent 'quasi-random' integration patterns into transcriptional start sites of active genes, as was seen in a gene therapy clinical trial for X-linked Severe Combined Immunodeficiency in France [7]. It was concluded that HIV-based 'lentiviral vectors' would be better candidates for permanent and more efficient gene transfer into HSCs as they integrate into resting cells and pose fewer risks by not integrating near transcriptional start sites of active genes.

Additionally, combination anti-HIV genes were developed that followed the same principle of combination ART drug therapy, inhibiting the HIV life cycle at multiple steps to prevent the generation of resistant viral strains. A major target for HIV gene therapy has been to knock down expression of the human protein CCR5, which is a chemokine receptor that HIV utilizes as a secondary receptor for attachment and entry into target cells. This rationale is based on a natural mutation in the *CCR5* gene of a small percentage of the human population. Individuals who are homozygous for this *CCR5* gene mutation are resistant to infection with CCR5-tropic strains of HIV [8].

Furthermore, the idea of a functional cure for HIV was postulated, underscored by another very interesting, anecdotal clinical case. An HIV-positive patient with leukemia was in need of an allogeneic bone marrow transplant to cure leukemia. The patient received an HSC transplant from a donor homozygous for the natural *CCR5* deletion. Leukemia was cured, and for the past 5 years, this patient has not had an HIV viral load while maintaining full withdrawal of ART. It seems that the transplantation of HIV-resistant HSCs was able to generate a new immune system capable of controlling HIV replication [9].

> "...HIV remains a major public health problem with no cure or preventative vaccine."

Clinical trials using lentiviral vectors for the transfer of anti-HIV genes have recently been conducted and the safety of this approach has been demonstrated [10]. However, improved gene transfer efficiency and a selective survival advantage of gene transduced cells still need to be achieved, as this will be the key for a working and permanent treatment for HIV using stem cell gene therapy.

Currently, our group has developed a novel triple combination anti-HIV vector that prevents HIV integration and has been shown to be safe and efficacious in an HIV *in vivo* humanized mouse model [11]. This vector contains two molecules acting prior to HIV integration, a CCR5 small hairpin RNA that mimics the phenotype observed with the natural *CCR5* deletion and a human/rhesus macaque and TRIM5α that is also based on natural resistance to HIV infection in Old World monkeys. The third molecule is a small RNA TAR decoy that inhibits transcriptional upregulation of HIV proviral DNA and prevents new virions from forming in infected cells. Our translational studies have progressed to the stage where we have designed a clinical protocol for a new HIV stem cell gene therapy clinical trial encompassing novel aspects of high-titer vector production, higher transduction efficiencies and cryopreservation methods that promise higher gene expression in the peripheral blood, as demonstrated in our *in vivo* HIV model [12]. A selective survival advantage of anti-HIV gene expressing cells will also be studied. This proposed clinical trial has already been presented to the NIH Recombinant DNA Advisory Committee that approved it to be presented to the US FDA, the final regulatory agency.

It is the authors' belief that steady improvements in the field of stem cell gene therapy for HIV will make it possible to repeat the result obtained with the patient who received a bone marrow transplant with naturally HIV-resistant bone marrow

stem cells, as long as enough transduced cells can be transplanted into patients. This will enable the replacement of an HIV-infected patient's immune system with one that is capable of blocking further HIV replication. Over time, it is hypothesized that viral reservoirs will be diminished due to the lack of HIV-infectable cells in the body from their expression of anti-HIV genes. With the insertion of anti-HIV genes, autologous stem cells will offer a marked improvement since combination genes can be applied to reducing the risk of generating resistant mutants while removing the risk of graft-versus-host disease. These critical improvements in the field hold the potential for developing a functional cure for HIV-infected patients.

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The authors have no 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. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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