

Gene therapy of Severe Combined Immunodeficiency (SCID)

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Severe combined immunodeficiencies (SCID) consist in a group of rare diseases characterized by a genetically determined block in T lymphocyte development. They represent an exquisite model to assess the feasibility of gene therapy for a series of reasons listed below. SCID are lethal before the age of one year in the absence of therapy; available therapy, i.e allogeneic hematopoietic stem cell transplantation has a significant risk of failure when performed in a non HLA geno identical setting; genes and associate pathophysiology have been unraveled for most SCID; it is expected that gene transfer into bone marrow progenitors should result in a major growth selective advantage of T cell precursors; finally once matured T cells have a very long life span. Based on this rationale, gene therapy of SCID has been developed. Methodology consists of ex vivo, retrovirally mediated gene transfer into CD34(+) hematopoietic progenitors harvested in the bone marrow. Usage of cytokines to induce CD34(+) cells to enter cell cycle (enabling provirus integration into the genome) and a fibronectin fragment to increase frequency of infection have led to define a protocol which permits to transduce 20-40 % of CD34(+) cells with a limited number of transgene copies per cell (1 on average). This approach has been so far successfully been utilized in 2 SCID conditions, i.e SCID-X1 (γ c cytokine receptor subunit deficiency) and adenosine deaminase (ADA). Altogether, 16 patients with typical SCID-X1 and 6 with ADA deficiency have been treated within the last 6.5 years. In 15/16 SCID-X patients treated by 2 groups and 6/6 deficient patients gene transfer led to the development of T lymphocytes. A cell dose threshold has been defined in the SCID-X1 trial, since in patients who received $< 3 \times 10^6$ CD34 (+) γ c(+)/kg, T cell reconstitution was only partial whereas it was complete in the others. Not only T cell counts normalized but also subsets distribution, repertoire and function. Consequently, efficient in vivo T cell immunity found to be sustained now over 6 years provides a protection against infections, enabling patients to live healthy in a normal environment, including school attendance.

Analysis of transgene integration sites by the usage of LAM-PCR method led to know that mature T cells stemmed from a limited set of transduced progenitors (order of magnitude : hundred) and that some multipotent hematopoietic progenitors have been transduced, a finding which provides hope for long term correction of this SCID condition.

To be efficient, however, gene therapy requires a) that transduced cells are not trapped into the spleen as observed in one patient with massive splenomegaly and b) that thymic function is preserved. Indeed after a limited number of years, thymic function is lost in SCID patients because of lack of cross talk between T cell precursors and epithelial cells, as observed in recipients of allogeneic HSCT in the absence of stem cell engraftment and in SCID patients with a leaky phenotype. Attempts of gene therapy of SCID-X1 in 2 patients in both of these settings (one each) failed despite effective gene transfer into CD34(+) cells.

Gene therapy carries the risk of inducing a serious adverse effect as observed in 3 patients with SCID-X1. In these 3 cases, integration of the provirus in the locus of a protooncogene (LMO-2) led to aberrant expression of LMO-2, triggered by the viral Long Terminal Repeat (LTR) an uncontrolled clonal T cell proliferation ensued. It eventually resulted in a leukaemia-like disease that became clinically detectable 30-36 months after gene therapy. Although it has been possible to destroy the clonal cells by chemotherapy in 2/3 cases, these SAE deserve cautious analysis in order to take lessons for the future. It became clear in the last years that retroviruses do not integrate in the genome randomly but with a high preference for genes and more specifically gene transcribed into targeted cells. Many of these genes in hematopoietic progenitors exert function in cell survival/proliferation therefore behave as protooncogenes. Since their expression provides a growth advantage to transduced cells, it is clear that the risk of insertional mutagenesis is higher than previously thought. It is however likely that additional risk factors should be involved including a role for the therapeutic transgene that can also be a protooncogene because it is a growth inducing factor (as γc), a role for an increased number of cycling progenitors in the bone marrow of young patients with SCID and possibly a role for genetic susceptibility factors.

In any case, future application of gene therapy, at least for SCID conditions requires reducing the risk of insertional mutagenesis. The usage of self inactivated (SIN) vectors (retro or more likely lentiviruses) should provide a way to preserve efficacy while preventing most of the risk because the enhancer activity of the viral LTR has been prevented by a deletion in the U3 region of the 3'LTR.

In the absence, so far of reliable prediction experimental models, further carefully designed gene therapy trials for SCID will ultimately be telling. In this context, extension of gene therapy to other SCID conditions such as Rag-1/-2, Artemis deficiencies as shown in experimental conditions, can be envisaged as well as the treatment of the Wiskott-Aldrich syndrome.

Long term future may be seen with the possibility to target integration in silent genomic sites by using specific integrases or to directly repair genes by using engineered nucleases to create site specific DNA breaks. Despite some advances, these attractive methodologies still carry a number of issues to be addressed (including among others non specific integration/recombination events), before clinical application can be envisaged.

In conclusion, efficacy of gene therapy for SCID has been reproducibly demonstrated, with a sustained effect for more than 6 years. This provides a basis to improve the methodology to increase its safety and allowing its extension to the treatment of a higher number of inherited diseases of hematopoiesis.

SCID as optimal models for gene therapy

- Lethal conditions
- Available therapy (allogenic hematopoietic stem cell transplantation) partially unsuccessful)
- Genes and pathophysiology known
- Gene transfer into progenitors expected to provide a growth selective advantage
- Mature cell product, i.e T cells have a long life span (> 10 years)