Gene therapy for primary adaptive immune deficiencies

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Gene therapy has become an option for the treatment of 2 forms of severe combined immunodeficiency (SCID): X-linked SCID and adenosine deaminase deficiency. The results of clinical trials initiated more than 10 years ago testify to sustained and reproducible correction of the underlying T-cell immunodeficiency. Successful treatment is based on the selective advantage conferred on T-cell precursors through their expression of the therapeutic transgene. However, "first-generation" retroviral vectors also caused leukemia in some patients with X-linked SCID because of the constructs' tendency to insert into active genes (eg, proto-oncogenes) in progenitor cells and transactivate an oncogene through a viral element in the long terminal repeat. These elements have been deleted from the vectors now in use. Together with the use of lentiviral vectors (which are more potent for transducing stem cells), these advances should provide a basis for the safe and effective extension of gene therapy's indications in the field of primary immunodeficiencies. Nevertheless, this extension will have to be proved by examining the results of the ongoing clinical trials. (J Allergy Clin Immunol 2011;127:1356-9.)

Key words: *Gene therapy, severe combined immune deficiency, retrovirus, lentivirus, Wiskott-Aldrich syndrome*

Abbreviations used

ADA:	Adenosine deaminase
HSCT:	Hematopoietic stem cell transplantation
LMO-2:	LIM domain only 2
LTR:	Long terminal repeat
NK:	Natural killer
PID:	Primary immunodeficiency
SAE:	Serious adverse event
SCID:	Severe combined immunodeficiency
SCID-X1:	X-linked severe combined immunodeficiency
SIN:	Self-inactivating
TCR:	T-cell receptor
WAS:	Wiskott-Aldrich syndrome
WASP:	Wiskott-Aldrich syndrome protein

There are many reasons that gene therapy has been developed in the field of primary immunodeficiencies (PIDs) over the last 20 years. Many PIDs are life-threatening conditions, notably severe combined immunodeficiencies (SCIDs) affecting T-cell development and function, Wiskott-Aldrich syndrome (WAS), hemophagocytic lymphohistiocytosis, innate immune deficiencies (eg, chronic granulomatous disease or Mendelian susceptibility to mycobacterial disease), and inherited autoimmune syndromes. The remarkable progress in treating PIDs has mostly been based on allogeneic hematopoietic stem cell transplantation (HSCT).[1] However, this approach is far from perfect, and serious adverse events (SAEs) can still occur (eg, graft-versus-host disease). In particular, graft-versus-host disease can damage the thymus and compromise the reconstitution of T-cell immunity. The limitations of HSCT are necessarily more pronounced in patients who lack HLA-compatible donors. Conversely, the success of HSCT provides a rational basis for the autotransplantation of transduced stem cells, the current approach in gene therapy for PIDs. Most PIDs display Mendelian inheritance, so that introduction of a normal copy of the mutated gene into the patient's cells should (in principle) be effective. The fact that disease-related genes have now been found for most PIDs[2] makes gene therapy a feasible approach for many of these conditions.

For some PIDs (eg, T-cell immunodeficiencies), it has become clear that transduced precursor cells can have a selective growth advantage. In several T-cell PIDs, the occurrence of somatic mutations positively modifies the mutated genes and leads to the development of functional T cells; the observed attenuation of disease phenotypes strongly supports this concept. This growth advantage is based on (1) the tremendous ability of T-cell precursors in the thymus to divide in an IL-7–dependent manner and after expression of the pre–T-cell receptor (pre-TCR), (2) positive selection, and (3) the very long lifespan of mature T cells. One can thus expect a few transduced T-cell precursors to give rise to a full, stable T-cell pool in a given subject. Hence SCID is considered to be an optimal model for assessing the feasibility of gene therapy.

GENE TRANSFER TECHNOLOGY

In the meantime significant advances in viral vector technology have enabled the transduction of dividing cells and thus replication of the transgene in progeny cells. Replication-defective retroviral vectors have been based on murine oncoretroviruses (the γ retrovirus), simian and human lentiviral viruses, spumaviruses, and transposons.[3] These vectors are generated in packaging cell lines transfected with (1) vector constructs containing sequences required for genome integration, the encapsidation sequence, the gene of interest, and various regulatory sequences and (2) constructs encoding the viral genes provided in trans to build replication-incompetent viral particles. A key advance was the creation of self-inactivating (SIN) viruses in which the absence of enhancer elements in their long terminal repeats (LTRs) makes them less able to transactivate endogenous genes after genome integration (see below).[4] In the absence of enhancers,

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TABLE I. T-cell primary immunodeficiencies: gene therapy trials

	Trial period	No.	Results	Trial status
SCID-X1 (Paris)	1999-2002	10	Eight alive, 4 SAEs	Closed
SCID-X1 (London)	2002-2009	10	Ten alive, 1 SAE	Closed
SCID-X1 (France/UK/US)	2010-present	3	Three alive	Open
SCID-ADA (Milan)	2000-2010	15	Fifteen alive, 13 off ERT	Open
SCID-ADA (London)	2004-2010	7	Seven alive, 3 off ERT	Open
SCID-ADA (US: 2 trials)	2007-2010	9	Nine alive, 5 off ERT	Open
WAS (Hanover)	2007-2010	10	Ten alive, 1 SAE	Closed
WAS (Milan)	2010-present	2	Alive	Open
WAS (France/UK/US)	2010-present	2	Alive	Open

ERT, Enzyme replacement therapy; *SCID-X1*, X-linked severe combined immunodeficiency; *UK*, United Kingdom; *US*, United States.

several internal promoters can be used to drive transgene transcription. Culture conditions for the transduction of hematopoietic progenitor cells have been improved by selecting the best cytokine cocktails and promoting virus/cell interaction through the addition of fibronectin fragments.

GENE THERAPY IN PATIENTS WITH SCIDS

After the advent of this vector technology, clinical trials were successfully initiated for typical X-linked SCID (SCID-X1; γc deficiency) in 1999 and then adenosine deaminase (ADA) deficiency. To date, gene therapy results are available for 20 patients with a typical SCID-X1, 5 patients with atypical SCIDs, and 31 patients with ADA deficiency (Table I).[5-10]

The SCID-X1 trials were associated with clinical events caused by vector genotoxicity and shall be discussed first.

Genotoxicity in SCID-X1 trials

Five of the 20 patients (4 in the Paris trial and 1 in the London trial) had T-cell leukemia 2 to 5.5 years after gene therapy.[10,11] After chemotherapy, 4 patients survived and showed sustained remission and T-cell immunity (see below).[7] One patient died from refractory leukemia.[10] In all cases it was found that the abnormal clone had 1 or 2 provirus integrations within a proto-oncogene locus. Many other genomic abnormalities were found.[10,11] Accordingly, the clinical trials were discontinued. Considerable effort was then devoted to investigating the mechanism underlying these SAEs. It was clearly shown that retroviruses do preferentially integrate within genes, especially actively transcribed genes. Epigenetic signatures that favor retroviral integration have been recently identified.[12,13] It turned out that the LIM domain only 2 (*LMO2*) locus in hematopoietic progenitors contains several of the features that favor frequent local integration. In parallel it became clear that the viral LTRs' enhancer activity could permanently turn on transcription of the target gene and thus trigger the leukemic process.[10,11] It is noteworthy that despite the use of a similar gene transfer technology in the ADA trials, none of the successfully treated patients (n = 21) had leukemia, a result that significantly differs from that of the SCID-X1 trials.[14] These findings strongly suggest that 1 or more disease-associated factors interfere with retroviral integration, such as the nature of progenitor cells in the bone marrow above the differentiation block, the possibly convergent effects of transgene and oncogene expression, and an inadequate *in vivo* milieu for cell growth (because of the inhibitory effects of purine accumulation in patients with ADA deficiency). The fact that a similar, *LMO2*-associated leukemic event was recently observed in a patient with WAS treated with *ex vivo* retrovirally mediated gene transfer into CD34 cells also indicates that the ADA deficiency setting should be regarded as unfavorable for the occurrence of leukemia.

Researchers have made huge efforts to construct safer vectors with the development of enhancer-deleted LTR-SIN vectors containing an internal promoter. This type of vector has been shown to be less genotoxic in *in vitro* assays of the clonogenicity of myeloid precursors.[4,15,16] Despite efforts to set up predictive *in vivo* assays in murine models, an absolute demonstration of safety can only be provided by the ongoing, recently initiated clinical trials. Furthermore, use of insulators (for functional isolation of the integrated provirus from the genomic environment) and addition of a suicide gene might be useful. Nevertheless, these measures will probably be only partially effective and have their own pitfalls. The use of HIV-derived lentiviral vectors might constitute an additional safeguard because this type of vector only integrates into genes (and not upstream of the transcription start site). This advantage might, however, be counterbalanced by greater transduction efficacy and thus more frequent vector integration into the patient's cells. Other potential improvements for the future include gene targeting to neutral ("safe harbor") genome regions and gene repair with target-specific nucleases.[17,18]

Efficacy in the SCID-X1 trial

At present, 18 of 20 patients with SCID-X1 treated in the Paris/London trials are alive 3 to 11.5 years after treatment (median, 8.2 years). Seventeen patients show the sustained presence of transduced lymphocytes.[5-7] Blood T-cell counts are in the normal or close-to-normal range, whereas phenotype and functional characteristics are also satisfactory. Remarkably, most patients (including the 4 who received chemotherapy) have some naive T cells, strongly suggesting the presence of ongoing, long-term thymopoiesis from transduced progenitor cells.

Gene therapy based on the development of T-cell immunity provided clear-cut clinical benefits to these patients because they can now deal normally with infections and are doing well in the absence of any therapy (apart from immunoglobulin substitution in some cases, see below). Long-term natural killer (NK) cell reconstitution is not as impressive, with only a few such cells in their blood (as is also observed after allogeneic HSCT in the absence of myeloablative conditioning). These results suggest that NK cell dynamics (precursor expansion, progeny lifespan, or both) differ significantly from T-cell dynamics. The patients' B-cell functions have been partially restored, despite very low (and decreasing) transduced B-lymphocyte counts. Accordingly, approximately half of the patients do not require immunoglobulin substitution. This observation might be due to (1) competition with normal B-cell development in the absence of γc expansion/function and (2) B-cell dynamics. It might well be of value to establish whether plasma cells in the bone marrow express γc. Thanks to the development of novel methods and technologies (eg, ligation-mediated PCR with multiple restriction enzymes and deep sequencing), a wealth of information has been provided through