Gene Therapy for Adenosine Deaminase Deficiency

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KEYWORDS

- Immune deficiency • Adenosine deaminase
- Gene therapy • Haematopoietic stem cell
- Reduced-intensity conditioning regimen

Primary immunodeficiency diseases (PIDs) are a genetically heterogeneous group of inherited disorders that affect distinct components of the innate and adaptive immune system, with impairment of their differentiation and/or functions.[1,2] Severe combined immunodeficiencies (SCIDs) represent about 15% of PIDs, ranging between 1:75,000 and 1:100,000 live births.[3] Adenosine deaminase (ADA) deficiency is a rare autosomal recessive disease belonging to the SCID group[4,5] (OMIM#102700). ADA deficiency represents the cause of approximately 10% to 20% of all cases of SCIDs,[4,6,7] with an overall prevalence in Europe that can be estimated to range between 1:375,000 and 1:660,000 live births, equivalent to 0.026 to 0.015 every 10,000 live births. Since 1972, ADA-SCID became the first immunodeficiency for which a specific molecular defect was identified, at both genetic and biochemical level.[8] ADA is a ubiquitous intracellular enzyme of purine metabolism. It catalyzes the irreversible deamination of adenosine (Ado) and deoxyadenosine (dAdo) in the purine catabolic pathway. Its deficiency results in metabolic toxicity because of the impairment of purine metabolism that leads to the intracellular accumulation of metabolic substrates, deoxyadenosine-X-phosphate (dAXP) and Ado. These metabolites are highly toxic for the cells, especially for lymphocytes and their precursors.[6] The human ADA gene is located on the long arm of chromosome 20 (20q12-q3.11).[7] Mutations in the ADA gene cause alterations in enzyme's activity, stability, and survival, leading to accumulation of Ado, dAdo, and adenine deoxyribonucleotides (dAXP) in plasma, red blood cells, and

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tissues. About 70 known different mutations have been identified in the ADA gene.[7,9,10] The main features of the disease are impaired differentiation and functions of T, B, and natural killer (NK) cells; recurrent infections; and failure to thrive. In addition, nonimmunologic abnormalities occur as the consequence of the systemic metabolic defect as a result of the accumulation of purine toxic metabolites.[4,5,11] Similar to other SCIDs, ADA-SCID is a fatal disease that usually leads to death in the first year of life, if not treated. However, among the SCIDs, it is one of the most difficult to handle clinically because of the concomitant systemic metabolic toxicity, which is typical of the disease (**Fig. 1**).

At present, the different therapeutic options available for its treatment are hematopoietic stem cell transplantation (HSCT), enzyme replacement therapy (ERT) with polyethylene glycol–modified ADA (PEG-ADA), and gene therapy (GT).

HEMATOPOIETIC STEM CELL TRANSPLANTATION

HSCTs for ADA-SCID represent about 11% of total transplants for SCID performed in Europe.[12] HSCT from an HLA-identical family donor is the gold standard treatment for patients with ADA-SCID, but it is available only for a minority of patients.[12] When feasible, this therapeutic procedure has a favorable outcome, with an overall 3-year survival of 81% in the European Society for Immunodeficiencies registry, with most recent transplant survival rates exceeding 90%, if HSCTs are performed promptly.[13–15] An overview of patients with ADA-SCID recruited in European and North American centers showed after matched sibling and matched family donor

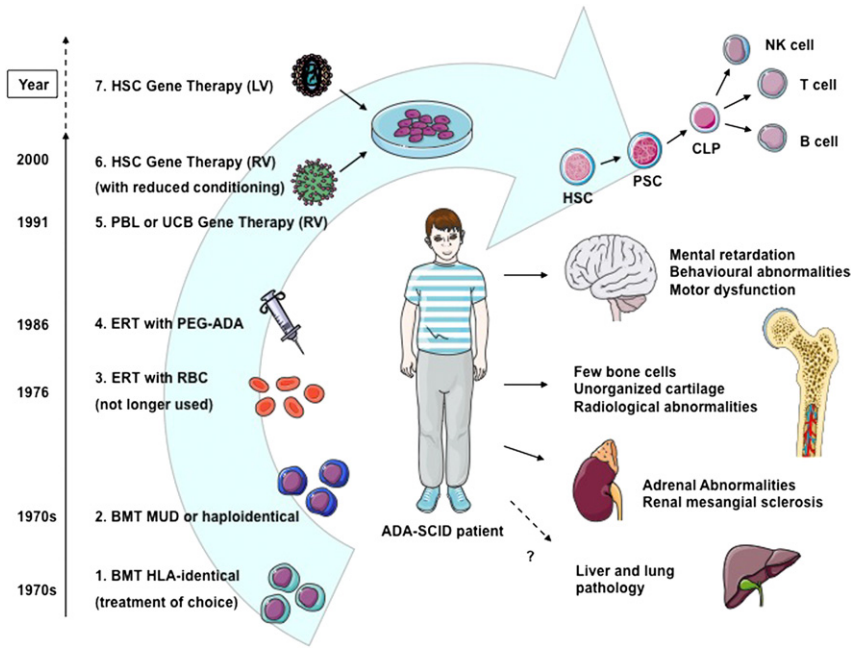

Fig. 1. Development of therapeutic options in ADA-SCID and specific immune and nonimmune features of the disease. (*From* Sauer AV, Aiuti A. New insights into the pathogenesis of adenosine deaminase-severe combined immunodeficiency and progress in gene therapy. Curr Opin Allergy Clin Immunol 2009;9(6):496–502; with permission.)

(often available due to the high incidence of consanguineous pedigrees) HSCTs an overall 1-year survival of 87% and 88%, respectively.[15] This type of HSCT is usually given without conditioning to reduce the risk of chemotherapy-associated toxicity. It usually results in a split chimerism, with only T lymphocytes of donor origin and other lineages, including B cells, remaining of host origin.[12,16] This may lead to variable correction of B-cell deficiency and of the metabolic defect.[16] In fact, dAdo purine metabolites, even if dramatically reduced compared with untreated patients, remain often higher than normal values (average dATP, 100 nmol/mL),[17] and plasma levels of Ado are persistently high, apparently without adverse effects. In addition, several cases in which neurologic complications of ADA-SCID are not corrected by the transplant have been described.[18]

HSCT FROM UNRELATED AND ALTERNATIVE DONORS

Transplants from matched unrelated donors (MUDs) or from umbilical cord blood (UCB) have been introduced in the past years as potential options for ADA-SCIDs. These transplants require typically chemotherapy and immunosuppressive drugs to accomplish complete donor chimerism in all cell lineages and long-term immune reconstitution. The ideal conditioning regimen has not been defined yet, with different groups using different approaches. Bone marrow or mobilized peripheral blood donors are searched worldwide in the bone marrow registries, but only less than 50% of the patients will find a fully matched donor. UCB units are readily available, and the degree of matching is less strict, being based on 6 alleles only (HLA-A, -B, -DR). However, so far, there is little available information on the outcome of MUD or UCB transplants for ADA-SCID in Europe. Recently, Gaspar and colleagues[15] showed a survival of 67% after fully matched unrelated donor transplants. Moreover, surviving MUD HSCT patients showed neurologic disturbances and late onset behavioral problems with developmental delay, typical of patients with ADA-SCID.[18,19] Cumulative data from unrelated UCB transplants in patients with primary immunodeficiencies showed a 5-year survival rate of 70%,[20] and all surviving patients presented complete immunologic reconstitution, but data on UCB transplants in patients with ADA-SCID are very limited.[15]

In conclusion, in the absence of an HLA-identical family donor, bone marrow transplantation for patients with ADA-SCID remains a treatment with a high risk of death. This is mainly because of a significant treatment-related toxicity, especially in MUD HSCT performed with conventional conditioning.

As an alternative source, child's parents' HSCs have also been used with the advantage that a donor is always and immediately available. However, this procedure is associated with many drawbacks, such as the increased risk of rejection and graft-versus-host disease (GVHD) or infections (due to the need of T-cell depletion). Data from a recent review show after mismatched unrelated and mismatched family donor transplants (mainly haploidentical) a survival of only 29% and 43% respectively, with most deaths in the first few months after transplant.[15]

Because of the encouraging experience in other forms of SCID, the Duke Center, USA, has pursued HSCT from T-cell–depleted parental bone marrow early in life and without conditioning to avoid toxicity of high-dose chemotherapy.[21] However, in ADA-SCID, this type of transplant is less effective than in other forms of SCID, with only 7 of 19 patients treated achieving engraftment of donor T lymphocytes.[15]

ENZYME REPLACEMENT THERAPY

ERT was introduced for the first time as lifesaving, noncurative treatment in 1986 for patients lacking an HLA-compatible donor.[22,23] The rationale for ERT is based on

the concept that maintaining high ADA activity in plasma, a weekly or twice-weekly intramuscular injection of PEG-ADA, eliminates Ado and dAdo derived from nucleotide and nucleic acid turnover. This protects immature lymphoid cells from apoptosis triggered by dAdo-induced dATP pool expansion, and from other mechanisms, restoring protective immune function in most patients in approximately 2 to 4 months.[23] Systemic ERT may also prevent metabolic toxicity to other organs, which may cause hepatic and neurologic dysfunction in some ADA-deficient patients. At present, updated data on 185 patients treated with PEG-ADA through September 2008 have been collected.[15] Approximately 20% of patients had died while on therapy, whereas 20% and 8% had discontinued ERT to undergo a potentially curative procedure such as HSCT and GT, respectively. Half of the deaths on ERT occurred within the first 6 months (40% in the first month), resulting from conditions present at diagnosis. The overall probability of surviving 20 years on ERT is estimated to be 78%. A patient alive 6 months after starting ERT had approximately 90% probability of surviving the next 12 years. Life-threatening adverse effects of ERT include refractory hemolytic anemia, chronic pulmonary insufficiency, and lymphoproliferative disorders and, rarely, hepatocellular carcinoma and infections.[5,15,23] Moreover, there is general agreement about the inadequacy of the immunologic reconstitution produced by PEG-ADA in a large fraction of patients on the long term (10–15 years). This is, at present, its biggest limitation.

GENE THERAPY

In the last decade, experimental GT approaches have been developed as successful alternative strategies.[24–26] Current GT approaches are based on the insertion of a healthy copy of the ADA gene into HSCs, although in the initial studies mature lymphocytes were also used as target cells. The ADA complementary DNA is transferred to the cells by the use of viral vectors that stably integrate into the human genome and transmit the therapeutic gene to the progeny of HSCs. The rational for GT resides on several potential advantages over ERT and HSCT.[25] Because it is an autologous procedure, transplantation of gene-corrected HSCs is potentially applicable to all patients, independent from the availability of a donor, with no delay for donor search. Moreover, the use of autologous gene-corrected stem cells avoids rejection and GVHD because of HLA mismatches or minor antigen incompatibility. Finally, GT does not require the use of immunosuppressive prophylaxes or high-dose conditioning regimens associated with organ toxicity (liver, lung, kidney, central nervous system), prolonged period of myelosuppression, and increased risks of infections.

Moreover, GT may be sufficient to definitively treat a patient, thus avoiding the need for lifelong ERT supplementation and its high burden in terms of costs and patients' quality of life. Furthermore, there are several evidence from preclinical and clinical observations that intracellular ADA delivered by engineered HSC or healthy donor transplant is more effective than exogenously administered ADA by ERT.[27]

Several clinical studies have investigated the safety and efficacy of ADA gene transfer into autologous hematopoietic cells using retroviral vectors. In the initial trials, 19 patients received infusions of transduced lymphocytes or hematopoietic progenitor cells.[27–33] No toxicity was observed, and in most patients, transduced T cells persisted in the circulation several years after infusion. However, the low gene transfer efficiency and engraftment levels observed in these patients did not allow to achieve a significant correction of the immunologic and metabolic defects, and all patients continued ERT.

However, this substitutive treatment might have abolished the selective growth advantage for gene-corrected cells. This hypothesis was confirmed by the observation of the case of 1 patient who discontinued PEG-ADA after showing inadequate immune reconstitution after ERT and repeated infusion of gene-corrected mature lymphocytes.[31] After ERT withdrawal, T cells containing the normal ADA gene progressively replaced the untransduced cells, resulting in restoration of normal T-cell functions and antibody responses to neoantigen. However, infusion of mature T cells was not sufficient to allow full correction of the metabolic defect, likely because of the limited mass of detoxifying cells.[31]

Rationale for Patients' Conditioning in Gene Therapy

Nonmyeloablative conditioning for patients with SCID undergoing allogeneic HSCT has shown that mainly T-lymphocyte line cells of the healthy donor engraft long term, whereas most or all B-lymphoid line cells usually do not engraft, and the other hematopoietic (myeloid, erythroid) lines remain those of the host. Also, the recent results obtained by the authors' group[26,34] and other researchers with SCID GT[32,33] indicate that in the absence of myeloablative therapy, engineered progenitors of T lymphocytes and mature T lymphocytes carry a selective advantage for growth that enables them to prevail over-diseased, nontransduced cells. However, engraftment levels of engineered B-lymphoid line cells and other hematopoietic cells are very low and do not reach therapeutic levels. This could be attributed to the presence of resident progenitor cells (B cells, myeloid cells, HSCs) competing in the bone marrow niche.

In particular, the experience throughout the years with GT of ADA-SCID has highlighted the potential and limitations of reconstitution limited to the T-cell lineage. On the other hand, the accumulation of toxic metabolites of ADA in lymphoid organs inhibits the development and growth not only of T lymphocytes but also of B lymphocytes, suggesting that, at the level of differentiating cells, gene-corrected cells within the B-cell lineage should carry a selective advantage once progenitors have engrafted. Furthermore, there is indirect evidence that the presence of particularly high levels of these metabolites could damage nonlymphoid organs also. The degree of metabolic control that can be achieved by the mass of cells able to produce the ADA gene and the correction of the defect of the B-lymphoid line and of the other hematopoietic lines are therefore the critical factors for the success of the approach based on GT. Thus, chemotherapeutic conditioning before transplanting engineered HSCs could be a key factor for a complete and persistent success of ADA-SCID GT by allowing the engraftment of multipotent progenitors.

A second objective of chemotherapeutic conditioning is to remove the ADA defective cells, when they are responsible for concomitant disorders such as autoimmune manifestations.

HEMATOPOIETIC STEM CELL GENE THERAPY

Autologous HSCs have been considered the optimal target cells for long-term, full correction of the ADA-SCID defect. In the past 10 years, more than 30 infants with ADA deficiency have been treated using retroviral vectors in Italy (The San Raffaele Telethon Institute for Gene Therapy, Milano), United Kingdom (Great Ormond Street Hospital, London), United States of America (Childrens Hospital of Los Angeles [CHLA]–National Institutes of Health [NIH]), and Japan (Hokkaido University, Sapporo).[26,33–37] Number of treated patients per center and type of vector and conditioning regimen used are shown in **Table 1**. Results on the first 10 patients treated in Milano have been recently reported.[26] Patients received, after a busulfan-based conditioning

Table 1
Clinical trials of hematopoietic stem cell gene therapy for ADA-SCID conducted in the last decade

Study	No of Patients Treated	Retroviral Vector	Conditioning Regimen	PEG-ADA Discontinuation (Yes/No)
HSR-TIGET[26,34]	15	GIADAI	Busulfan (4 mg/kg)	Yes
GOSH[15,17]	5	SFFV-ADA-WPRE	Melphalan (140 mg/m^2)	Yes
CHLA-NIH 1[33,35]	4	GCsap-M-ADA and MND-ADA	No	No
CHLA-NIH 2[33,35]	6	GCsap-M-ADA and MND-ADA	Busulfan (75–90 mg/m^2)	Yes
Hokkaido[36]	2	GCsap-M-ADA	No	Yes

Abbreviations: HSR-TIGET, The San Raffaele Telethon Institute for Gene Therapy, Milano, Italy; MND, myeloproliferative sarcoma virus [MPSV] enhancer, negative control region deleted, dl587rev primer binding site substituted; SFFV, spleen focus-forming virus.

protocol (4 mg/kg i.v.), a high dose of CD34$^+$ cells (mean 8.2 $\times$ 10^6 CD34$^+$ cells/kg), containing an average of 28.6% of transduced progenitors. Levels of transduction were similar to those of other studies, but the large number of CD34$^+$ cells infused in most patients may have been important for the success of this study. High levels of gene marking were seen in peripheral T (88% average marking), B (52%), and NK (59%) cells (**Fig. 2**). Moreover, the detection in peripheral blood and bone marrow of ADA-transduced cells in multiple lineages (myeloid, erythroid, megakaryocytic) demonstrated the efficacy of the reduced-intensity conditioning regimen in achieving substantial HSC engraftment (see **Fig. 2**). Nine patients have benefited from GT, showing effective adequate systemic detoxification, reduction in the frequency of infection, and improvement of their weight-height growth curve. Moreover, a progressive reconstitution of T-cell counts and functions was also observed, although at slower rate with respect to standard HSCT.[26] Five patients showed complete immune reconstitution with discontinuation of intravenous immunoglobulin and humoral immune responses to vaccinal and microbial antigens (**Fig. 3**). On the other hand, 1 patient who experienced autoimmunity during ERT showed an insufficient engraftment and, because of the recurrency of autoimmunity, continued to require steroid treatment.[26]

Five additional patients have been treated with promising results. At present, all 15 patients treated with this protocol are alive, and only 2 patients have required ERT after GT.[37]

In a similar trial performed in London using an alternative retroviral construct (viral spleen focus-forming virus–long terminal repeat promoter for ADA gene transcription) and a single-dose melphalan conditioning regimen, 5 patients have been treated (see **Table 1**).[15,17] Of the 5 treated patients, 2 have shown very good immune reconstitution and are clinically well, 1 patient restarted PEG-ADA, and 2 patients failed GT because of a poor stem cell harvest and a low-level stem cell transduction efficiency, respectively. In the 2 most successfully reconstituted patients, ADA expression was observed in different hematopoietic lineages, including red blood cells, leading to effective metabolic control. In both patients, recovery of thymopoiesis was demonstrated after GT. Gene correction was proved in most T cells and NK cells, whereas significant gene marking was also observed in granulocytes and monocytes.

Encouraging results have also been recently reported in similar studies performed at the NIH and CHLA (see **Table 1**).[15,35] In a first study, 4 patients received GT without

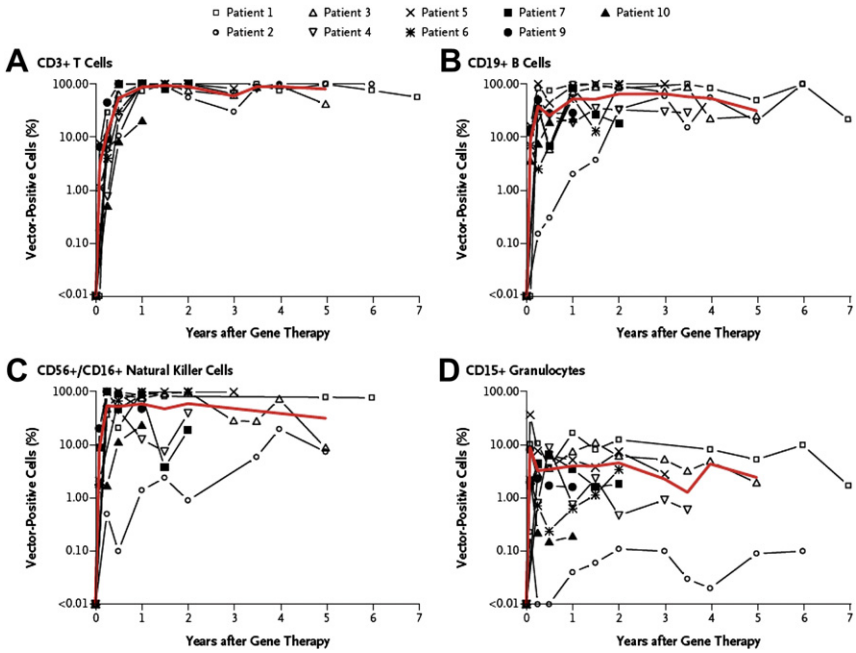

Fig. 2. Persistence of ADA-transduced cells in different lineages after gene therapy. The proportions of vector-positive cells (on a log10 scale) for 9 patients with ADA-SCID treated with gene therapy and on average (*red line*) are shown for different cell lineages from peripheral blood samples: $CD3^+$ T cells (panel *A*), $CD19^+$ B cells (panel *B*), $CD56^+/CD16^+$ NK cells (panel *C*), and $CD15^+$ granulocytes (panel *D*). (*From* Aiuti A, Cattaneo F, Galimberti S, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 2009;360(5):447–58; with permission.)

conditioning, leading to low levels of engraftment and no sustained immunologic improvement while continuing ERT.[33] In a second clinical trial, 6 patients were treated with low-dose busulfan in combination with PEG-ADA withdrawal. Two patients with a follow-up longer than 1 year showed a normalization of in vitro T-cell function and improvement of immunoglobulin production, in 1 case associated to normal responses to vaccines. In all but 1 case, production of ADA by peripheral blood mononuclear cells resulted in sustained detoxification of purine metabolites at levels comparable to those observed after HSCT.[15] One patient with a pre-GT cytogenetic abnormality experienced a prolonged cytopenia after busulfan conditioning.[15]

An alternative GT strategy based on PEG-ADA withdrawal without myeloablative conditioning was attempted on 2 patients with ADA-SCID in Japan. Preliminary reports have shown some degree of immunologic reconstitution, at lower level than in patients pretreated with conditioning, but a longer follow-up is required for full evaluation of this approach (see **Table 1**).[36]

All together, these results showed the efficacy of infusion of autologous ADA gene–corrected HSCs in combination with a reduced-intensity conditioning regimen.

SAFETY OF ADA-SCID GENE THERAPY

A potential risk associated with gene transfer can be represented by ‘‘insertional oncogenesis,’’ by which a retroviral vector may land into the genome adjacent to cellular genes, such as proto-oncogene, leading to inappropriate expression of the

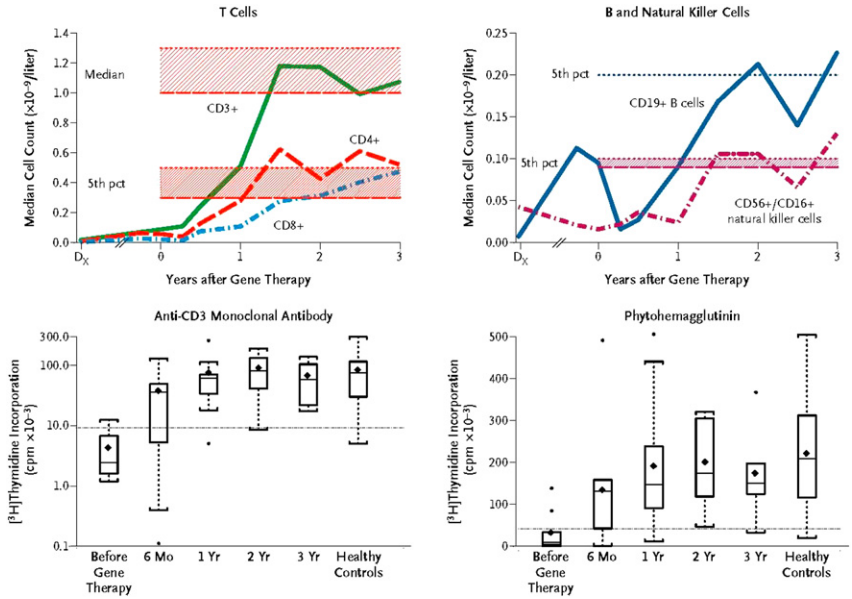

Fig. 3. Immune reconstitution after gene therapy. Upper panel shows the median cell counts for $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells (*left*) and B cells and NK cells (*right*) after gene therapy. Reference values for age are also shown as shaded areas or dotted lines.[26] Bottom panel shows data for the in vitro proliferative responses to anti-CD3 monoclonal antibody (on a log10 scale, *left side*) and to phytohemagglutinin (on a linear scale, *right side*). The data are expressed as counts per minute (cpm) in ADA-deficient patients and in healthy controls. The dashed horizontal line represents the 5th percentile for healthy controls. (*From* Aiuti A, Cattaneo F, Galimberti S, et al. Gene therapy for immunodeficiency due to ADA deficiency. N Engl J Med 2009;360(5):447–58; with permission.)

neighboring gene. This event may lead to clonal proliferation and eventually leukemic proliferation, as observed in 5 patients with SCID-X1 who developed a T-cell leukemia and 2 patients with chronic granulomatous disease who manifested with myelodysplasia due to monosomy 7, 31 to 68 months after GT.[38,39]

However, in addition to vector-mediated activation of cellular genes, it is believed that other factors including the disease background, the nature of the transgene, and the acquisition of other genetic abnormalities unrelated to vector insertions are also needed for aberrant expansion. So far, the cumulative experience of GT for ADA-SCID did not reveal the occurrence of clonal expansion or leukemic proliferation, indicating that it has a favorable risk-benefit profile. This is in agreement with the finding of a polyclonal pattern of T-cell receptor repertoire and vector integrations in treated patients.[26,40]

Moreover, in vitro studies on transduced clones generated ex vivo from patients with ADA-SCID, several years after GT, failed to show significant signs of perturbation of neighboring genes and did not lead to growth advantage or alteration in cellular behavior.[40]

PERSPECTIVES FOR NOVEL GENE TRANSFER APPROACHES

For future applications in the context of ADA-SCID and other inherited primary immunodeficiencies, the use of self-inactivating human immunodeficiency virus

(HIV)–derived lentiviral vectors may improve the safety and efficacy of gene transfer into HSCs. These vectors offer a unique combination of advantages over retroviral vectors because they integrate efficiently into HSCs, allow stable and robust transgene expression, and significantly alleviate the safety concerns associated with retroviral vector integration. In addition, they can be adapted to contain physiologic cellular promoters rather than viral promoters used for retroviral vectors. Lentiviral vectors have recently entered the clinic with wide-ranging applications, as several trials are ongoing or are beginning in Europe and the United States to treat HIV infection,[41] neurodegenerative syndromes,[42] primary immunodeficiencies,[43] or genetic diseases such as thalassemia.

The efficacy of lentiviral vector–mediated ADA gene transfer has been recently explored in preclinical mouse models of ADA deficiency using 2 different strategies. In the first approach, murine HSCs transduced with lentiviral vectors transplanted into ADA-deficient mice resulted in full metabolic detoxification, restoration of ADA activity, and differentiation and immune functions of lymphoid cells.[44] Pretransplant irradiation was crucial for long-term survival of ADA−/− mice because animals receiving transplants without irradiation died 2 weeks after transplantation due to poor engraftment. In a different approach, a SIN–lentiviral vector was used to treat neonatal ADA−/− mice directly by intravenous injection.[45] In addition to prolonged survival, mice showed significantly increased lymphoid cell counts and reconstitution of T-cell proliferation, although no selective advantage of gene-corrected T cells was observed.

Alternative GT approaches based on sophisticated system that allow gene correction or gene editing[46,47] could represent a further improvement in safety over integrating vectors, but their clinical application requires further optimization and testing at preclinical level.

SUMMARY

In the last decade, GT has been developed as a successful alternative strategy for patients affected by ADA-SCID lacking an HLA-identical sibling donor. This approach has been shown to be well tolerated and efficacious. The introduction of a reduced-intensity conditioning regimen has been identified as a crucial factor in achieving adequate engraftment of HSCs and therapeutic levels of ADA. The future development of novel vector technology, such as lentiviral vectors, might provide a superior efficacy and safety profile. The prospects for extending the application of GT to a broader spectrum of genetic diseases, including primary immune deficiencies, remain strong.

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