



Ten years of gene therapy for primary immune deficiencies

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Gene therapy with hematopoietic stem cells (HSC) is an attractive therapeutic strategy for several forms of primary immunodeficiencies. Current approaches are based on ex vivo gene transfer of the therapeutic gene into autologous HSC by vector-mediated gene transfer. In the past decade, substantial progress has been achieved in the treatment of severe combined immunodeficiencies (SCID)-X1, adenosine deaminase (ADA)-deficient SCID, and chronic granulomatous disease (CGD). Results of the SCID gene therapy trials have shown long-term restoration of immune competence and clinical benefit in over 30 patients. The inclusion of reduced-dose conditioning in the ADA-SCID has allowed the engraftment of multipotent gene-corrected HSC at substantial level. In the CGD trial significant engraftment and transgene expression were observed, but the therapeutic effect was transient. The occurrence of adverse events related to insertional mutagenesis in the SCID-X1 and CGD trial has highlighted the limitations of current retroviral vector technology. For future applications the risk-benefit evaluation should include the type of vector employed, the disease background and the nature of the transgene. The use of self-inactivating lentiviral vectors will provide significant advantages in terms of natural gene regulation and reduction in the potential for adverse mutagenic events. Following recent advances in preclinical studies, lentiviral vectors are now being translated into new clinical approaches, such as Wiskott-Aldrich Syndrome.

Primary immunodeficiencies (PID) are rare genetic disorders of the innate and adaptive immune system. Over 120 different gene mutations have been identified which cause impairment in the differentiation and/or functions of immune cells with different degrees of severity.¹ Transplant of hematopoietic stem cells (HSC) from an HLA-identical sibling donor is the treatment of choice for severe combined immunodeficiencies (SCID) and other types of PID with poor prognosis. Despite substantial improvements in the procedures for allogeneic HSC transplant, some patients continue to experience long-term complications after transplant,² and the use of alternative donors is still associated with high morbidity and mortality.³ Gene therapy could represent a valid alternative to HSC transplantation when the genetic mutation of the patient has been identified. Current gene therapy approaches are based on ex vivo gene transfer of the therapeutic gene into autologous HSC by means of gene transfer using non-replicating viral vectors.⁴

The Rationale for Gene Therapy in PID

Gene therapy has several potential advantages over allogeneic transplant approaches,^{4,5} including a rapid access to treatment and the lack of graft-versus-host disease

(GVHD) and of complications associated with standard conditioning regimens. On the other hand, gene transfer approaches based on integrating vectors possibly risk insertional mutagenesis,⁶ which is dependent on the type of vector employed, the disease background, and the nature of the transgene.

The first efforts to treat a PID with gene therapy were made in the early 1990s, when gene corrected peripheral blood lymphocytes or HSC from bone marrow or umbilical cord blood were infused into patients affected by adenosine deaminase (ADA)-deficient SCID. These initial attempts were limited by an insufficient gene transfer into long-lasting gene-corrected progenitors.^{4,7,8} Subsequently, the improvement in gene transfer technology and experimental design allowed the successful treatment of SCID due to IL2RG deficiency (SCID-X1) and ADA-SCID with a single infusion of genetically corrected HSC. A crucial element for this success was the selective advantage provided by the therapeutic genes in transduced lymphocytes. Such an advantage had been previously observed in transplanted SCID patients³ and in PID patients who carried lymphocyte clones re-expressing the mutated protein by reversion of mutations to normal sequences.⁹ Since this advantage is not

present at the HSC level, it was only after the inclusion of a reduced intensity conditioning in the clinical trial for ADA-SCID gene therapy¹⁰ that substantial levels of multilineage engraftment were achieved.

Since 1991, gene therapy has been investigated at the preclinical level in several PID and over 90 patients have been treated with gene therapy (**Table 1**). Here we will discuss the experience obtained in the past 10 years of clinical gene therapy approaches for ADA-SCID, SCID-X1, and chronic granulomatous disease (CGD) and present the recent advances in the clinical development of gene therapy for another form of PID, Wiskott-Aldrich syndrome (WAS).

Gene Therapy for ADA-deficient SCID

ADA-deficiency is a SCID variant characterized by impaired T, B, and NK cell development and functions, recurrent infections and failure to thrive. In addition, non-immunological abnormalities have been described in several organs as result of the accumulation of purine toxic metabolites.¹¹ Allogeneic transplant from mismatched related donors is affected by a higher morbidity and mortality as compared with other SCID variants.¹¹ ADA-SCID children who lack a compatible donor are often treated with enzyme replacement therapy (pegylated bovine ADA, PEG-ADA).¹¹ PEG-ADA results in clinical improvement and metabolic correction, but the immunological reconstitution is often incomplete and lifelong treatment is very expensive.¹¹

The pilot clinical studies showed that the infusion of transduced lymphocytes or hematopoietic progenitors was safe and feasible.^{4,7,8} Transduced T cells persisted in the circulation several years after infusion in the majority of

patients, but this was not sufficient to achieve significant clinical benefit. In patients receiving gene-corrected HSC, the frequency of vector-transduced cells remained below a therapeutic threshold. Moreover, immune functions were not proven to be sustained by gene-corrected cells, since all patients continued to receive enzyme replacement therapy. Withdrawal of PEG-ADA favored the selective accumulation of gene-corrected T cells,^{12,13} leading to improved immune responses in one patient,¹³ but transduced T cells were not sufficient to allow adequate systemic detoxification.

A major improvement was obtained after the introduction of a reduced dose of intravenous busulfan (4 mg/kg) prior cell reinfusion to make space in the bone marrow for gene corrected HSC¹⁰ and allow multilineage reconstitution and ADA expression. Since the year 2000, 15 patients without HLA-identical sibling donors have been treated with transduced autologous bone marrow CD34⁺ cells according to this experimental protocol (**Table 2**)¹⁴ (and Aiuti and Roncarolo, unpublished data). Enrolled children had shown an inadequate response to PEG-ADA or had failed an haploidentical transplant. To exploit the selective advantage for ADA-expressing cells in a toxic environment, enzyme replacement therapy was not administered after gene therapy. The outcome of the first 10 patients treated at HSR-TIGET has been recently described.¹⁴ Busulfan induced a transient myelosuppression without organ toxicity, which allowed stable and efficient engraftment of transduced HSC at levels ranging from 1% to 10%.¹⁴ The dose of infused CD34⁺ cells and their frequency of transduction were important parameters in determining the proportion of gene corrected HSC engrafting in patients. After gene therapy, vector-ADA cells were detected in all myeloid and lymphoid subsets, the latter being more represented due to their survival advantage. The identification of shared vector integrants among distinct hematopoietic lineages demonstrated the long-term engraftment of multipotent clones of HSC.¹⁵ Vector-derived ADA was expressed in lymphocytes, monocytes, bone marrow cells and erythrocytes, allowing an efficient systemic detoxification up to 8 years after gene therapy. Nine out of the 10 patients displayed recovery of polyclonal thymopoiesis, substantial increase in T-cell counts and normalization of their functions, including susceptibility to apoptosis and proliferative responses to mitogens and antigens.¹⁶ Clonal analysis of long-term repopulating cell progeny revealed polyclonal T-cell populations, with more than 100 different transduced progenitors contributing to T lymphopoiesis.¹⁵ Evidence of specific antibodies to vaccination antigens and pathogens was obtained in 5 children who discontinued IVIg treatment. The progressive reconstitution of immune and metabolic functions led to significant improvement of patients' development and protection from severe infec-

Table 1. Preclinical and clinical gene therapy studies for primary immune deficiencies (PID).

Preclinical studies	Clinical studies
SCID due to deficiency of	ADA-SCID
-RAG-1	SCID-X1
-RAG-2	CGD
-Artemis	JAK3-SCID
-IL7R	LAD
-PNP	WAS
SAP-deficiency	
CD40L deficiency	
X-linked agammaglobulinemia	
IPEX	

CGD indicates chronic granulomatous disease; LAD, leukocyte adhesion deficiency; IPEX, Immune dysfunction, polyendocrinopathy, enteropathy, X-linked.

Table 2. Summary of the clinical experience of hematopoietic stem cell (HSC)-gene therapy for adenosine deaminase-deficient severe combined immunodeficiencies (ADA-SCID) in the last decade.

Study	No. pts treated	Conditioning	Longest follow-up	Efficacy	Toxicity
HSR-TIGET ^{11,14}	15	Busulfan (4 mg/kg)	8 y	Yes	No
GOSH ^{11,19}	5	Melphalan (140 mg/m ²)	5.5 y	Yes	No
CHLA/NIH ¹⁷	4	No	8 y	No	No
CHLA/NIH ^{11,17,18}	6	Busulfan (75-90 mg/m ²)	2 y	Yes	Pancytopenia due to pre-existing cytogenetic abnormality (1 pt)

GOSH indicates Great Ormond Street Hospital; CHLA, Children's Hospital Los Angeles.

tions, without adverse events related to gene therapy. At present all patients are alive and only 2 patients have required enzyme replacement after gene therapy.

In a clinical trial conducted recently in the US, busulfan conditioning and withdrawal of PEG-ADA also led to improved immunological and metabolic outcome^{11,17} (Table 2). However, one patient experienced a prolonged cytopenia following busulfan conditioning, as consequence of a pre-existing cytogenetic abnormality, pointing out at a potential limitation for patients subject to autologous gene transfer.¹⁸ Efficient metabolic and immunological correction was also reported in ADA-SCID patients who underwent gene therapy combined with a single dose of melphalan as conditioning regimen^{11,19} in the UK (Table 2).

None of the ADA-SCID patients enrolled in the different clinical trials worldwide showed adverse events related to insertional mutagenesis, indicating that gene therapy for ADA-SCID has a favorable risk-benefit profile. Taken together, these results indicate that HSC gene transfer with nonmyeloablative preconditioning is now an option to be considered for any ADA-SCID patient lacking an HLA-identical sibling donor. The clinical development of lentiviral vectors encoding ADA^{20,21} might further improve gene therapy approaches for this disease.

Gene Therapy for SCID-X1

IL2RG deficiency (SCID-X1) is caused by mutations in the common cytokine receptor gamma chain (γ c) gene, which is

part of the receptors for IL-2 and five other cytokines (IL-4, IL-7, IL-9, IL-15, and IL-21). SCID-X1 patients lack T and NK cells, while B cells are present but functionally impaired.⁵ Despite the high survival rate after HLA-identical allogeneic transplant, some patients display persistent defective humoral or cellular immune functions.⁵

The first clinical gene therapy protocol was conducted at Hôpital Necker (Paris) and was based on ex vivo γ c gene transfer using retroviral vectors into autologous CD34⁺ bone marrow cells²² (Table 3). Progenitor cells were then re-infused into patients, who lacked a matched sibling or unrelated donors, in the absence of any preparative conditioning. A similar study was carried out at Great Ormond Street Hospital (GOSH, London).^{23,24} This protocol varied from the French study essentially in the use of GALV-pseudotyped instead of a conventional amphotropic MLV-based vector. Overall, 17 out of the 20 SCID-X1 patients enrolled in both clinical trials benefited from gene therapy.⁵ In nearly all patients T-cell counts reached normal levels and became functionally competent, as demonstrated by normal responses to mitogens and specific antigens. Ten years of follow-up available for the patients who were first to be treated have provided evidence for still active thymopoiesis, with broadly diversified TCR repertoire.^{5,25} The finding that virtually all T and NK cells but fewer B cells and myeloid cells carried the transgene clearly demonstrates that γ c expression confers to T and NK progenitors a strong selective growth advantage. Nevertheless, several patients discontinued immunoglobulin

Table 3. Summary of the clinical experience of hematopoietic stem cell (HSC)-gene therapy for SCID-X1.

Study	No. pts treated	Longest follow-up	Efficacy	Toxicity
Hôpital Necker ^{22,25}	10	10 y	Yes	T-cell leukemia (4 pts)
GOSH ^{23,24}	10	7 y	Yes	T-cell leukemia (1 pt)
GOSH, Necker, ²⁷ NIH ²⁶	5 (age 10-20)	3 y for pts with engraftment	No, improvement in 1 pt	No

GOSH indicates Great Ormond Street Hospital.

infusions and showed antigen-specific responses following vaccination. When gene therapy was attempted in older SCID-X1 patients, this resulted in inability to recover T-cell immunity in most patients^{26,27} (**Table 3**). The failure to reactivate thymopoiesis could likely be related to the age of the patients at the time of intervention and/or the clinical history of chronic infection and GVHD. This phenomenon probably reflects a crucial time-dependency of the ability to restore effective thymopoiesis, since the prolonged absence of interaction between thymocytes and thymic epithelial cells can cause irreversible thymic hypoplasia. These data support the recommendation that gene therapy in SCID patients should be considered as early as possible after diagnosis.

The success of SCID-X1 gene therapy was limited by the occurrence of serious gene transfer-related toxicity. Four patients in the French trial and one in the English trial developed clonal T-cell proliferation that became evident 2 to 6 years after treatment. This leukemia-like disease was the result of vector-mediated up-regulation of host cellular oncogenes by the MLV LTR.^{25,28,29} Gammaretroviral vectors naturally insert into active genes, and the strong enhancer elements found in the viral LTR can transactivate neighboring promoters leading to aberrant gene expression. In the cases of SCID-X1 it is believed that an initial aberrant expression of an oncogene (mainly LMO2) led to proliferation of specific clones and the addition of other genetic events, eventually resulting in leukemic transformation. Chemotherapy allowed sustained remission in 4 cases, and these patients continued to benefit from gene therapy, but 1 patient died due to refractory leukemia.^{25,29} The occurrence of these severe adverse events led to the development of a new area of research focused on virus-mediated oncogenesis and significantly contributed to improving safety standards for gene therapy vectors.

Gene Therapy for Chronic Granulomatous Disease

The encouraging results of the gene therapy trials for SCID demonstrated the curative potential of gene transfer and provided a rationale for the development of gene therapy protocols for other immunodeficiencies, such as CGD. This PID is an inherited disorder of innate immunity in which phagocytic leukocytes are unable to generate superoxide and related toxic oxygen metabolites to kill invading bacteria and fungi by respiratory burst.³⁰ Consequently, affected patients are susceptible to recurrent opportunistic bacterial and fungal infections, leading to the formation of chronic granulomas. Approximately 70% of CGD patients carry defects in the X-linked gene encoding for gp91phox (X-CGD), which is part of the NADPH oxidase complex. Although lifelong antibiotic prophylaxis reduces the

incidence of infection in CGD patients, the overall annual mortality is still high (2%-5%) and the success rate of HSC transplant is limited by GVHD and inflammatory flare-ups at infectious sites.^{30,31} Clinical gene therapy trials have been carried out in Frankfurt, London, Zurich and in the USA.³⁰ In the first clinical trials conducted at the US National Institutes of Health, patients demonstrated very low levels of reconstituted neutrophils for up to 6 months, without clinical benefit.³² In a subsequent study nonmyeloablative conditioning with busulfan (8 mg/kg) was administered before the infusion of genetically modified CD34⁺ cells from mobilized peripheral blood.³³ After a period of myelosuppression, cell counts recovered gradually and a substantial gene transfer (over 20%) was detected in both patients the neutrophils of both patients. This resulted in a large fraction of phagocytes expressing a functional GAPDH complex with significant clinical improvement. However, an unexpected clonal expansion of transduced myeloid cells occurred about 5 months post-treatment. Vector-transduced myelopoiesis expanded due to integrations activating the zinc finger transcription factor homologs MDS1/EVI1, PRDM16 or SETBP1. Both patients subsequently developed myelodysplasia with monosomy 7 and the first one died 2.5 years after gene therapy as a result of a severe sepsis.³⁰ The second patient has undergone successful allogeneic transplant. Surprisingly, gp91phox expression was almost completely abolished due to silencing of the promoter but not of the SFFV enhancer sequence. In another patient treated at the NIH, long-term persistence of marked cells (1%) and reduction in the number of infections have been observed.³¹ Despite the evidence of a transient therapeutic benefit in CGD patients, future clinical trials will require superior vector design to improve safety of gene transfer into HSC while ensuring adequate transgene expression in myeloid lineages.

Safety Issues and Development of Novel Technologies

Despite the occurrence of serious complications associated with gene transfer, the experience of gene therapy for PID in the past decade shows an overall survival superior to allogeneic transplant from unrelated or haploidentical donors. It still unclear why the risk of insertional mutagenesis is associated with specific diseases and/or clinical trials. In the case of the CGD trial, the use of a retroviral vector with SFFV LTR sequences, which contains potent enhancer elements for gene expression in HSC, likely favored the activation of specific genes and led to the observed clonal expansion. Several possible explanations may account for the different safety profile of the ADA-SCID trial with respect to SCID-X1. First, ADA is a constitutively expressed enzyme of purine metabolism while IL2RG is a signaling molecule receptor chain that induces cell

proliferation and is upregulated upon T-cell activation. Vector-derived common γ c may be expressed at inappropriate levels in different stages of differentiation, and it is interesting to note that the kinetics of T-cell reconstitution were substantially different in the two trials, the one of ADA-SCID being slower than the one observed for the SCID-X1 trials. Furthermore, synergistic mechanisms of tumorigenesis may result from the interaction of IL2RG with cellular proto-oncogenes activated by vector insertions, such as LMO2.³⁴ Finally, the SCID-X1 background may favor the accumulation of mutations or show a predisposition to oncogenesis as shown in two mouse models.^{35,36}

Most efforts to improve safety and efficiency for SCID gene therapy have focused on the development of new self-inactivated (SIN) gammaretroviral and lentiviral vectors containing cellular promoters, which carry a reduced risk of insertional mutagenesis. Potential candidates for future clinical trials for SCID-X1 include SIN-gammaretroviral vectors driven by the human EF1- α promoter³⁷ and SIN-lentiviral Vectors (SIN-LV) based on HIV-1 incorporating the ubiquitously acting chromatin opening element (UCOE).³⁸ These vectors induced stable γ c gene expression and fully restored lymphoid differentiation and functions. In the case of CGD, SIN lentiviral vectors encoding gp91^{phox} under a constitutive promoter restored the oxidase enzyme activity in a human/mouse xenograft model.³⁹ In several other PID (Table 1), studies in animal models, including disease-specific and xenogeneic models, have been completed successfully, providing the basis for a potential clinical development of gene therapy in other disorders.

Development of a Gene Therapy Approach for Wiskott-Aldrich Syndrome

WAS is another good candidate for a gene therapy approach. This disease is a severe X-linked immunodeficiency caused by mutations in the gene encoding for WASp, a key regulator of signaling and cytoskeletal reorganization in hematopoietic cells. Mutations in the WAS gene result in a wide array of clinical manifestations, ranging from X-linked thrombocytopenia (XLT) to a full-blown WAS phenotype characterized by thrombocytopenia, immunodeficiency, eczema, and increased susceptibility to tumors and autoimmune manifestations.⁴⁰ Gene therapy could represent a valid alternative for patients who lack an HLA-identical donor and particularly for older patients who are at risk of a poor outcome after allogeneic HSC transplantation.^{41,42} Preclinical and clinical evidence suggest that WASp-expressing cells have a proliferative or survival advantage over WASp-deficient cells, strongly supporting the development of a gene therapy strategy. Indeed, a selective accumulation of revertant T cells has been observed in many WAS patients

who underwent spontaneous reversion in a lymphoid progenitor.⁹ Preclinical studies for WAS gene therapy were initially based on retroviral vectors and then progressed to lentiviral vector approaches in order to increase efficacy and safety. *In vitro* studies on human WASp-deficient B and T cell lines transduced with oncoretroviral vectors demonstrated the restoration of proliferative response to anti-CD3 and cytoskeleton remodelling.^{43,44} Studies in *was*^{-/-} mice have shown that the injection of transduced murine HSC into lethally irradiated *was*^{-/-} recipient mice resulted in efficient engraftment and improvement in TCR-driven T-cell proliferation as well as cytokine production.^{44,45} In addition, amelioration of colitis and normalization of secondary immune response to influenza virus were observed after gene therapy in *was*^{-/-} mice.⁴⁵ Protocols for the application of WAS gene therapy are currently under development at several centers in Europe. The first gene therapy study has been initiated in 2007 in Germany to assess the feasibility, toxicity, and potential benefit of HSC gene therapy with an MLV-derived retroviral vector encoding WASp under the control of a viral promoter.⁴⁶ Preliminary results from the German trial seem to be encouraging, but the safety profile of the retroviral vector has still to be evaluated carefully. Since self-inactivating (SIN) vectors, including lentiviral vectors, have shown a reduced genotoxic potential as compared to gammaretroviral vectors,⁴⁷ we and others have developed a gene transfer approach based on a SIN lentiviral vector containing a minimal WAS promoter (1.6 kb) to target the expression of the therapeutic WAS transgene physiologically to the hematopoietic system.⁴⁸ This vector was shown to successfully restore WASp expression in CD34⁺ cells, B cells, T cells, and dendritic cells, and to correct TCR-driven activation in T-cell lines derived from WAS patients.^{49,50} Moreover, *in vivo* studies performed in two different *was*^{-/-} strains mouse model showed evidence of long-term multilineage WASp expression and the correction of immune, inflammatory and cytoskeletal defects.⁵¹ Long-term observation of a large group of mice treated with gene therapy did not display any severe adverse event related to gene transfer.⁵¹ These findings represent the necessary basis to move toward the implementation of a lentiviral vector-mediated gene therapy clinical trial.⁴⁸

Conclusions

In the last decade, transplantation of genetically corrected HSC has been developed as a successful alternative strategy for PID treatment. These trials have shown for the first time that gene therapy is an efficacious treatment for a severe genetic disorder. More than 30 SCID patients have benefited from gene therapy, achieving life-saving immune reconstitution lasting for up to 10 years after treatment. The use of the conditioning regimen has been identified as a

crucial factor in achieving therapeutic levels of gene-corrected, multilineage HSC, leading to superior engraftment in the myeloid lineages in the ADA-SCID as compared to the SCID-X1 trial. This finding has important implications for translating the experience of PID to other blood-borne disorders, such as lysosomal storage disorders and thalassemia, which require a higher therapeutic threshold.

The occurrence of serious complication in the SCID-X1 and CGD trials have highlighted the risks of insertional mutagenesis with retroviral vector technology. Advances in molecular genetics, vector design and HSC biology will favor the extension of clinical trials to other PID variants. Currently, several clinical trials are being developed with self-inactivating lentiviral vectors to treat various forms of PID, including SCID and WAS. The development of novel strategies based on zinc-finger nucleases that can correct specific DNA sequences is very promising⁵² but still far from clinical application.

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Conflict of interest disclosure. The authors declare no competing financial interests.

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