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## Gene therapy for adenosine-deaminase-deficient severe combined immunodeficiency

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Adenosine-deaminase-deficient SCID was the first inherited disease to be treated with gene therapy. This life-threatening disorder is characterized by a purine defect that leads to impaired immune functions, recurrent infections and systemic metabolic abnormalities. The early gene therapy trials showed the safety and feasibility of engineering haematopoietic stem cells and peripheral blood lymphocytes using retroviral vectors. However, all patients were maintained on enzyme-replacement therapy, which prevented the evaluation of its efficacy and abolished the selective advantage for gene-corrected cells. It is only recently that the clinical efficacy of gene therapy has been investigated in the absence of enzyme-replacement therapy. Results of these studies showed that gene therapy with peripheral blood lymphocytes allowed correction of the T-cell defect, but provided insufficient systemic detoxification. Gene transfer in bone marrow stem cells, associated with non-myeloablative conditioning, allowed full immunological and metabolic correction of the adenosine-deaminase defect with clinical benefit. These results have important implications for future applications of gene therapy in other blood-borne disorders.

**Key words:** gene transfer; haematopoietic stem cells; peripheral blood lymphocytes; primary immunodeficiency; adenosine deaminase; retroviral vector.

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Somatic gene therapy represents a promising therapeutic option for inherited disorders of the immune system. Severe combined immunodeficiency (SCID) due to adenosine-deaminase (ADA) deficiency was the first inherited disease to be treated with a gene transfer approach. The first clinical trials represented an important milestone for proving the safety and feasibility of gene therapy, but did not reach therapeutic levels. Recent trials designed to overcome the initial hurdles have produced crucial information on the role of genetically modified peripheral blood lymphocytes (PBLs) and haematopoietic stem cells (HSCs) in correcting the immune and metabolic defects, with the first demonstration of clinical efficacy for this disease. The knowledge and expertise deriving from these studies represent an important platform for the development of other gene transfer protocols for acquired or genetic disorders involving the haematopoietic system.

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## CHARACTERISTICS OF ADA-SCID

SCID diseases are fatal inherited disorders caused by mutations in crucial genes controlling the development and functions of immune cells. ADA deficiency is an autosomal-recessive disorder representing the cause of about 20% of SCID diseases.<sup>1-3</sup> ADA is an enzyme belonging to the purine metabolism pathway, expressed ubiquitously, that catalyses the conversion of adenosine into inosine. The ADA enzyme is predominantly intracellular, but a fraction is present on the cell surface of lymphocytes bound to CD26 molecules.<sup>4</sup> A lack of ADA is responsible for an increase in adenosine and 2'-deoxyadenosine in the plasma and an increase of nucleotides (mainly dATP) in lymphoid tissues, red blood cells (RBCs) and organs such as kidney and liver.<sup>5</sup> The intracellular accumulation of purine metabolites leads to impaired growth, differentiation and functions of immunocompetent cells through different mechanisms. There is a quantitative relationship between residual ADA activity and the metabolic and clinical phenotype.<sup>6</sup> Mild forms of the disease are also detected in adults, with predominance of the autoimmune manifestations.<sup>7</sup> In addition to the immune defect, there is increasing evidence that the accumulation of toxic metabolites may also cause alterations in non-lymphoid organs. Skeletal, renal, hepatic and neurological abnormalities have been described in some patients, indicating that the disease should be considered as a 'systemic' metabolic disorder.<sup>3,8,9</sup>

## CURRENT THERAPEUTIC OPTIONS AND PROBLEMS

Allogeneic transplantation of HSCs from an HLA-identical sibling donor is a safe and effective procedure, but this is only available for a minority of patients. Transplantation from haplo-identical or matched unrelated donors is affected by high morbidity and mortality due to infections or graft-vs-host disease, with an overall survival of less than 30%.<sup>10</sup> Moreover, HSC transplantation may result in variable correction of B-cell deficiency and of the metabolic defect<sup>11,12</sup>, with no significant improvement in the behavioural abnormalities observed in ADA-SCID patients.<sup>9</sup>

Weekly intramuscular injections of bovine ADA conjugated to polyethylene-glycol (PEG-ADA) have resulted in correction of metabolic abnormalities, improved growth and a decrease in the incidence of severe infections.<sup>2,3,13</sup> However, most patients remain lymphopenic and about 20% of patients fail to respond to the treatment. In addition, the therapeutic potential of PEG-ADA is limited by the high costs and the risk of developing neutralizing antibodies or autoimmunity.<sup>2,3</sup>

## THE RATIONALE FOR GENE THERAPY

The strong rationale for somatic gene therapy and the need for alternative treatments led to the design of clinical trials based on retroviral-mediated gene transfer of the normal ADA gene into autologous HSCs or PBLs.<sup>14</sup> Replication-deficient, recombinant retroviruses derived from the backbone of Moloney murine leukaemia virus (MMLV) were selected for these trials because of their safety records and the ability to efficiently insert the transduced gene into the genome of dividing haematopoietic cells. The marker gene encoding neomycin resistance (neoR) was usually contained in the vector to permit monitoring and selection of

transduced cells. Several lines of evidence supported the rationale for gene therapy with haematopoietic cells in ADA-deficient SCID.<sup>15</sup> First, the initial in-vitro gene transfer studies showed that expressing a normal ADA gene into deficient lymphocytes corrected their abnormal sensitivity to adenosine purine metabolites.<sup>16</sup> Second, the ADA gene is expressed ubiquitously and does not require regulation, and ADA enzymatic activity as low as 10% of normal is sufficient to allow normal immune functions. Finally, cells carrying a normal ADA gene have a selective growth advantage over deficient cells in haematopoietic cell transplantation.<sup>3</sup> These hypotheses were confirmed in preclinical models in which gene-corrected PBLs from ADA-SCID patients were injected into immunodeficient mice; the transduced PBLs survived in the long term and were endowed with survival advantage with respect to uncorrected ADA-deficient cells.<sup>17,18</sup> The selective growth advantage for corrected cells was also supported by the finding of T-cell clones that spontaneously reverted the ADA mutation in vivo in patients.<sup>19,20</sup> These preclinical results were instrumental for the approval of the first gene therapy protocols of ADA at the National Institutes of Health (NIH)<sup>21</sup> and at the Scientific Institute HS Raffaele in Milan.<sup>22</sup>

## EARLY GENE THERAPY STUDIES

Pilot trials initiated in the 1990s were based on the infusion of autologous PBLs and/or HSCs, genetically modified ex vivo by retroviral vectors that stably insert the ADA gene into the target cells and their progeny.<sup>23–25</sup>

### Gene therapy with PBLs

PBL gene therapy was proposed for the treatment of ADA deficiency as an alternative or in combination with HSC gene therapy, based on the finding that patients receiving a bone marrow (BM) transplant reached sufficient immune reconstitution by sole engraftment of donor T cells. Six ADA-SCID patients enrolled in three different protocols have been treated with multiple infusions of gene-corrected PBLs.<sup>23,24,26–29</sup> The three protocols employed similar retroviral vectors but differed in the method of T-cell activation, the duration of ex-vivo culture, and the dose of infused cells (Table 1).<sup>30</sup> The available follow-up of the treated patients extends from 6 years to over 12 years, with a cumulative observation time of almost 60 years; no adverse events or toxicity have been reported. Overall, a total of more than  $3 \times 10^{11}$  PBLs have been infused and the integrated vector persisted long after the patients stopped receiving treatment. In the first patient treated at NIH, 10 years after the last cell infusion, 20% of the PBLs still contained vector ADA and expressed enzyme activity at approximately 25% of normal levels.<sup>29</sup> Significant improvements in immune functions, including raises in PBL cell counts and normalization of proliferative responses to mitogens and antigens, were observed. However, all patients continued PEG-ADA, thus preventing assessment of the actual impact of gene therapy on the disease. Furthermore, it is likely that the systemic delivery of the ADA enzyme abolished the growth advantage for cells carrying the normal ADA gene. This has been reported in patients carrying 'naturally' corrected T cells due to spontaneous reverse mutations, in whom the initiation of PEG-ADA caused a progressive decline of corrected T cells (Table 1).<sup>3,20</sup>

**Table 1.** Gene therapy trials for adenosine-deaminase-deficient severe combined immunodeficiency using peripheral blood lymphocytes.

Investigators	Patients	Gene transfer protocol	Other treatments
Blaese et al <sup>24,29</sup>	2	Transduction after stimulation with antiCD3 monoclonal antibody and IL-2	PEG-ADA
Bordignon et al <sup>23</sup>	2	Transduction after stimulation with PHA + IL-2	PEG-ADA
Onodera et al <sup>26</sup>	1	Same as Ref. <sup>24</sup>	PEG-ADA
Aiuti et al <sup>28</sup>	1	Same as Ref. <sup>23</sup>	PEG-ADA then discontinued

PEG-ADA, polyethylene glycol-adenosine deaminase; IL-2, interleukin-2.

### Gene therapy with HSCs

Since the design of the first gene therapy trials, HSCs have been considered to be the optimal target cells for long-term, complete correction of ADA deficiency.<sup>15</sup> Early gene therapy studies showed that retroviral-mediated transfer of the ADA gene into autologous BM<sup>23,31</sup> or umbilical cord blood (UCB) progenitors was feasible, resulting in engraftment and differentiation into multiple lineages.<sup>23,32</sup> However, the proportion of transduced cells was insufficient to achieve ADA expression at therapeutic levels. A recent study by Schmidt et al used molecular techniques to evaluate the number of genetically modified progenitors giving rise to mature T lymphocytes in one patient treated after birth with infusion of UCB CD34<sup>+</sup> cells.<sup>33</sup> In this study, the majority of transduced T lymphocytes originated from a single, prethymic, stem/progenitor cell<sup>33</sup>, persisting up to 94 months after gene therapy. The transduced T lymphocytes carried a diverse T-cell receptor (TCR) repertoire, and no signs of leukaemic transformation were observed. These data suggest that few long-lasting progenitor cells were transduced and engrafted in this ADA-SCID patient. As discussed above, this could be related to the lack of a selective pressure for the growth and expansion of gene-corrected cells, since all the patients continued to receive enzyme-replacement therapy.

### THE EFFECT OF PEG-ADA DISCONTINUATION ON PBL GENE THERAPY

Despite the rationale for discontinuation of enzyme-replacement therapy, it was difficult from an ethical point of view to justify cessation of a potentially beneficial treatment. Conditions for discontinuation were recognized in a patient who failed to maintain immune responses and who displayed clinical features of immune imbalance during PEG-ADA treatment.<sup>28</sup> During the initial tapering-down phase of PEG-ADA, the infusions of transduced T cells were intensified and then stopped. Shortly after PEG-ADA cessation, the large majority of PBLs became vector-transduced lymphocytes, indicating the presence of a selective advantage in a non-detoxified environment. T-cell counts increased to values significantly higher than those observed during enzyme-replacement therapy, and biochemical studies showed restoration of ADA enzymatic activity in T cells.<sup>28</sup> Immune correction was demonstrated by the development of normal in-vitro T-cell proliferative responses, as well as by primary and secondary antibody production after vaccination. During this period, the patient had normal growth and did not

experience severe infections. However, after discontinuation of PEG-ADA, the systemic purine metabolites increased, leading to a decrease in B-cell numbers and serum immunoglobulins. Thus, the mass of ADA-corrected T lymphocytes was insufficient to achieve adequate levels of detoxification. Taken together, these data show that PBL gene therapy can be efficacious as a single therapy in correcting the T-cell defect, but not the metabolic defect. Increasing the dose of PBLs and ADA expression might contribute to correct the metabolic disease in ADA-SCID patients.

### **STEM CELL GENE THERAPY COMBINED WITH LOW-INTENSITY MYELOABLATION RESULTS IN CORRECTION OF IMMUNE AND METABOLIC DEFECTS**

Overall, these clinical studies highlighted the importance of developing more efficient and rational gene transfer approaches based on HSCs. We therefore designed an improved clinical protocol that attempted to overcome the limitations of the early trials by: (i) adopting an improved gene transfer procedure to increase the number of transduced HSCs infused; (ii) favouring HSC engraftment with low-intensity conditioning; and (iii) providing a selective advantage to gene-corrected cells in the absence of PEG-ADA.<sup>34</sup>

Based on recent advances in the field of retroviral-mediated gene transfer into HSCs<sup>35–38</sup>, several improvements were introduced in the design of the gene transfer protocol. The retroviral supernatant was produced under conditions optimized for human CD34<sup>+</sup> cell gene transfer.<sup>39</sup> The culture medium included the growth factors FLT3 ligand, stem cell factor, thrombopoietin and interleukin-3, a combination that was found to be optimal for gene transfer into HSCs from ADA-SCID patients, and allowed maintenance of their differentiation capacity into myeloid, natural killer (NK), B and T cells (Ficara et al, manuscript submitted). The retroviral vector was preloaded on a surface coated with a recombinant fibronectin fragment (Retronectin)<sup>40</sup> to facilitate the co-localization of cells and retroviral particles. The total length of ex-vivo manipulation of the BM CD34<sup>+</sup> cells was 4 days, which included the first day of prestimulation with cytokines, followed by 3 days of transduction with the retroviral vector.

Busulfan was chosen as the single chemotherapeutic drug because of its wide use in HSC transplantation for paediatric patients<sup>41</sup>, including primary immunodeficiencies. The proposed dosage (2 mg/kg for two consecutive days) represented about 25% of the typical dosage used in full myeloablative regimens, that usually comprise additional drugs. The rationale for the use of a non-myeloablative regimen derived from gene-marking studies in animal models and recent experience in BM transplantation for haematological disorders. When non-myeloablative regimens were used in the context of allogeneic transplant for the congenital haematological disorders, sufficient levels of donor engraftment were achieved with no major toxicity.<sup>42</sup> Animal studies found that sustained marking was achieved at relevant levels in both lymphoid and myeloid cells using an improved gene transfer protocol and non-myeloablative conditions.<sup>43,44</sup>

Previous studies indicated that gene-corrected cells only acquire a selective growth advantage in the absence of PEG-ADA. Therefore, we chose to recruit patients who did not have access to enzyme-replacement therapy. This allowed us to evaluate the efficacy of gene therapy alone.

Two ADA-SCID patients who did not have an HLA-identical sibling donor were enrolled in this new gene therapy protocol.<sup>34</sup> Patient 1 (aged 7 months) received one log higher BM CD34<sup>+</sup> cells/Kg compared with Patient 2 (aged 30 months), containing a similar proportion of transduced progenitors. Neither of the patients experienced

toxicity or required transfusions, and after a transient myelosuppression, haematopoiesis recovered as expected.

Following infusion of autologous transduced BM CD34<sup>+</sup> cells, a remarkable and sustained degree of marking (up to 10%) was observed in granulocytes as well as megakaryocytic, erythroid and CD34<sup>+</sup> progenitors. The engraftment of multipotent engineered HSCs was also proven by the consistent finding of a significant proportion of BM CD34<sup>+</sup> cells that contained the ADA vector and retained the ability to repopulate the human lymphoid system after secondary transplant in the SCID-hu mouse model. The highest levels of engraftment were observed in T, B and NK cells (up to 100% of transduced cells), confirming that, in the absence of systemic detoxification, gene-corrected lymphocytes could gain the strongest selective advantage over untransduced cells. Gene therapy resulted in normalization of PBL counts in Patient 1 and improvement in Patient 2, with normalization of in-vitro T-cell proliferative responses in both patients.<sup>34</sup> Restoration of thymic activity was demonstrated by the increase in phenotypically naive T cells and by the appearance of T-cell receptor excision circles in the peripheral blood. Serum immunoglobulin levels improved and production of specific antibodies after antigen vaccination was observed in Patient 1, allowing intravenous immunoglobulins (IVIG) infusions to cease. Biochemical studies demonstrated the production of ADA enzymatic activity in lymphocytes, as well as in other haematopoietic cells, such as myeloid cells and RBCs. This was paralleled by an important decline in RBC toxic dAXP metabolites and a substantial amelioration of systemic toxicity. Two additional patients have been treated recently with similar results.<sup>45</sup> All patients have remained clinically well, free from severe infections, with normal growth and development, and off PEG-ADA.<sup>34,45</sup> Furthermore, no adverse events have been observed, with the longest follow-up now at over 3 years. Collectively, these results show that gene therapy with engineered HSCs is efficacious in correcting both the immune and metabolic defects of ADA deficiency with documented clinical benefit (Table 2).

**Table 2.** Gene therapy trials for adenosine-deaminase-deficient severe combined immunodeficiency using haematopoietic stem cells.

Investigators	Patients	Gene transfer protocol	Other treatments
Bordignon et al <sup>23</sup>	2	Infection of BM mononuclear cells with viral supernatant, no cytokines added	PEG-ADA
Kohn et al <sup>25,32,33</sup>	3	Infection of UCB CD34 <sup>+</sup> cells with viral supernatant in the presence of cytokines (IL-3, SCF, IL-6)	PEG-ADA, discontinued briefly in one patient
Hoogerbrugge et al <sup>31</sup>	3	Co-culture of BM CD34 <sup>+</sup> cells on irradiated producer with IL-3	PEG-ADA, in one of the patients started after 4 months
Aiuti et al <sup>34,45</sup> (and unpublished data)	4	Infection of BM CD34 <sup>+</sup> cells with viral supernatant, in the presence of retronectin and cytokines (SCF, TPO, FLT3 ligand, IL-3)	No PEG-ADA; Low-intensity conditioning with busulfan
Candotti et al <sup>47,53</sup>	4	Infection of BM CD34 <sup>+</sup> cells with viral supernatant, in the presence of retronectin and cytokines (SCF, TPO, FLT3 ligand)	PEG-ADA; No conditioning

UCB, umbilical cord blood; BM, bone marrow; SCF, stem cell factor; TPO, thrombopoietin; IL-3, interleukin-3; IL-6, interleukin-6; PEG-ADA, polyethylene glycol-adenosine deaminase.

## SAFETY ISSUES

The cumulative results of PBL gene therapy in ADA-SCID show the safety of gene therapy with autologous T cells, considering the high number of infused T cells, their *in vivo* long-term persistence and the extended period of observation. The overall safety of retroviral-mediated gene transfer is further supported by recent results obtained in 31 patients affected by leukaemias or lymphomas who received transduced PBLs for controlling graft-vs-host disease after allogeneic BM transplantation.<sup>46</sup> None of the patients showed evidence of adverse events or toxicity related to gene therapy.

None of the ADA-SCID patients treated with HSC gene therapy in the early or recent trials have developed serious side-effects or toxicity.<sup>30,47</sup> Concerns regarding the safety of retroviral-vector-mediated gene therapy were raised after the report of two cases of uncontrolled lymphoid proliferation in SCID-X1 patients treated with gene therapy.<sup>48</sup> Activation of the LMO2 proto-oncogene at the site of vector integration played a key role in the development of leukaemia, but other factors may have contributed<sup>49,50</sup> including features of SCID-X1 patients, the effect of  $\gamma$ c transgene<sup>51</sup> or an abnormal proliferative advantage of corrected cells. In addition, there has been no other report of adverse events in more than 10 years of studies in large animal models and gene therapy clinical trials with retroviral vectors. In summary, these data suggest that the risk of insertional mutagenesis due to retroviral-mediated gene transfer in ADA-SCID remains low. Nevertheless, careful patient monitoring should be continued to assess the long-term safety of ADA-SCID patients treated with transduced HSCs.<sup>52</sup>

## DISCUSSION

### The role of PBL gene therapy

PBL gene therapy can restore normal T-cell ADA activity and functions if a selective advantage for transduced cells is created upon removal of systemic detoxification. However, PEG-ADA treatment is required for achieving adequate numbers of lymphocytes for *ex-vivo* gene transfer into PBLs.<sup>30</sup> Important limitations of PBL gene therapy include the inability to correct a previously skewed T-cell repertoire and to generate *de-novo* specificities<sup>29</sup>, as well as an insufficient correction of the systemic metabolic defect.<sup>28</sup>

### The use of conditioning to improve HSC engraftment

Several lines of evidence indicate that a certain degree of BM ablation is necessary for adequate engraftment of genetically modified HSCs in animal models. Nevertheless, concerns about chemotherapy-associated toxicity had prevented the use of conditioning in gene therapy trials for inherited disorders in humans.

Recently, four ADA-SCID patients have been treated with a different clinical protocol based on an improved gene transfer procedure for BM CD34<sup>+</sup> cells, without administering chemotherapy and continuing PEG-ADA injections. Results of the first year of follow-up showed very low levels of engraftment of marked cells, even in the lymphoid lineage, with no significant immune improvements, thus confirming the requirement for a selective advantage for the engraftment and expansion of HSCs and their lymphoid progeny.<sup>53</sup> In the gene therapy trials for SCID-X1, no conditioning was

administered prior to re-infusion of autologous engineered BM CD34<sup>+</sup> cells.<sup>54,55</sup> In nine of 10 patients treated with gene therapy, normal T- and NK-gene-corrected cells developed, allowing restoration of numbers, phenotype, repertoire and functions of T cells.<sup>48</sup> However, B lymphocytes and other haematopoietic cells remained mainly untransduced, and B-cell functions were variably corrected.<sup>54,55</sup>

The results of our ADA-SCID trial suggest that a low-intensity conditioning regimen can be safely administered to patients, allowing the stable engraftment of gene-corrected multilineage HSCs, resulting in a higher frequency of transduced myeloid cells and B lymphocytes. This is in agreement with the experience of BM transplants for SCID diseases, which showed that the use of a conditioning regimen increased the frequency of sustained engraftment and allowed more frequent engraftment of donor B cells and myeloid cells.<sup>3,56,57</sup> Finally, conditioning may also promote immunological tolerance of both the engineered cells and the proteins encoded by the vector, which is of particular relevance for the treatment of diseases in which the patients are immunocompetent.

### **Enzyme-replacement therapy and selective advantage**

Early gene therapy trials based on MMLV retroviral vectors showed poor transduction efficiency of HSCs. The results of the ADA-SCID study, as well as those of the SCID-X1 gene therapy trial, indicate that MMLV vectors are efficacious tools for gene transfer into human stem/progenitor cells when used in adequate gene transfer protocols. A major factor in the successful outcomes of these trials resided in the selective growth advantage conferred *in vivo* to transduced lymphocytes in the context of a SCID disease. Avoiding the use of enzyme-replacement therapy in the ADA-SCID trial<sup>34</sup> enabled evaluation of the clinical efficacy of gene therapy alone, and full exploitation of the selective advantage for gene-corrected cells. Indeed, in the absence of systemic detoxification, gene-corrected lymphocytes could gain the strongest selective advantage over untransduced cells. However, in haematological disorders lacking such an advantage, alternative strategies should be considered to increase gene transfer efficacy, transgene expression, HSC engraftment or *in-vivo* selection.

### **Further improvements are desirable**

Different degrees of engraftment and immune reconstitution were observed among ADA-SCID patients treated with gene therapy. In the second patient treated with HSCs and conditioning, the overall levels of engraftment and reconstitution were lower than in other patients. This finding might be explained by the lower dose of transduced HSCs transplanted and the reduced degree of host BM ablation observed in this patient.<sup>34</sup> Further improvements in vector design and gene transfer protocol, as well as optimization of the cell dose and conditioning, may ensure more consistent outcomes in the future.

## **SUMMARY**

The results from the ADA-SCID gene therapy trial represent a paradigmatic approach for gene transfer technologies based on retroviral vectors. The pilot studies showed the therapeutic potential and limitations of gene transfer into HSCs. Recent clinical

trials produced crucial information about the role of genetically modified PBLs and HSCs in correcting immune and metabolic defects, with the first demonstration of clinical efficacy for this disease.

Both engineered T lymphocytes and HSCs persisted in the long term in treated patients, and the absence of enzyme-replacement therapy conferred a marked selective advantage for gene-corrected cells of lymphoid lineage. However, only HSC-based gene therapy allowed full correction of both the immune and metabolic defects of ADA deficiency. These results also suggest that moderate conditioning can be safely administered to the patients in order to facilitate the engraftment of gene-corrected HSCs, and achieve higher levels of transduced myeloid cells and B-lymphocytes. A prolonged observation time in a larger cohort of patients will be required for full evaluation of long-term safety and efficacy of ADA-SCID gene therapy. The use of low-intensity conditioning associated with transplantation of gene-corrected HSCs may have important implications for the treatment of other genetic and acquired diseases, including primary immunodeficiencies, metabolic disorders, haemoglobinopathies, acquired immunodeficiency syndrome and malignancies.

#### Practice points

- gene transfer in HSCs results in immune and metabolic correction of ADA-deficient SCID with clinical benefit
- gene therapy is effective in the absence of enzyme-replacement therapy
- the use of low-intensity conditioning improves the engraftment of gene-corrected HSCs
- if results are confirmed in the long term, a single injection of gene-corrected stem cells may be sufficient for life-long treatment of a patient, at costs that are comparable with those of HSC transplantation

#### Research agenda

- improve vector design and gene transfer protocol
- identify optimal cell dose and conditioning regimen
- apply gene transfer approaches to other inherited or acquired severe disorders of the haematopoietic systems
- extend observation time and number of patients enrolled to assess long-term safety and efficacy of gene therapy

## REFERENCES

1. Parkman R, Gelfand EW, Rosen FS, et al. Severe combined immunodeficiency and adenosine deaminase deficiency. *New England Journal of Medicine* 1975; **292**: 714–719.
2. Hershfield MS. Adenosine deaminase deficiency: clinical expression, molecular basis, and therapy. *Seminars in Hematology* 1998; **35**: 291–298.
3. Hirschorn R. Immunodeficiency disease due to deficiency of adenosine deaminase. In Ochs H, Smith C & Puck J (eds) *Primary Immunodeficiency Diseases*. Oxford: Oxford University Press, 1999, pp 121–139.

4. Richard E, Arredondo-Vega FX, Santisteban I, et al. The binding site of human adenosine deaminase for CD26/dipeptidyl peptidase IV. The arg142gln mutation impairs binding to cd26 but does not cause immune deficiency. *Journal of Experimental Medicine* 2000; **192**: 1223–1236.
5. Carlucci F, Tabucchi A, Aiuti A, et al. Capillary electrophoresis in diagnosis and monitoring of adenosine deaminase deficiency. *Clinical Chemistry* 2003; **49**: 1830–1838.
6. Arredondo VF, Santisteban I, Daniels S, et al. Adenosine deaminase deficiency: genotype-phenotype correlations based on expressed activity of 29 mutant alleles. *American Journal of Human Genetics* 1998; **63**: 1049–1059.
7. Ozsahin H, Arredondo-Vega FX, Santisteban I, et al. Adenosine deaminase deficiency in adults. *Blood* 1997; **89**: 2849–2855.
8. Ratech H, Hirschhorn R & Greco MA. Pathologic findings in adenosine deaminase deficient-severe combined immunodeficiency. II. Thymus, spleen, lymph node, and gastrointestinal tract lymphoid tissue alterations. *American Journal Pathology* 1989; **135**: 1145–1156.
9. Rogers MH, Lwin R, Fairbanks L, et al. Cognitive and behavioral abnormalities in adenosine deaminase deficient severe combined immunodeficiency. *Journal of Pediatrics* 2001; **139**: 44–50.
- \* 10. Antoine C, Muller S, Cant A, et al. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968–99. *Lancet* 2003; **361**: 553–560.
11. Hirschhorn R, Roegner MV, Kuritsky L, et al. Bone marrow transplantation only partially restores purine metabolites to normal in adenosine deaminase-deficient patients. *The Journal of Clinical Investigation* 1981; **68**: 1387–1393.
12. Ochs HD, Buckley RH, Kobayashi RH, et al. Antibody responses to bacteriophage phi X174 in patients with adenosine deaminase deficiency. *Blood* 1992; **80**: 1163–1171.
13. Weinberg K, Hershfield MS, Bastian J, et al. Lymphocyte ontogeny in adenosine deaminase-deficient severe combined immune deficiency after treatment with polyethylene glycol-modified adenosine deaminase. *The Journal of Clinical Investigation* 1993; **92**: 596–602.
14. Bordignon C & Roncarolo MG. Therapeutic applications for hematopoietic stem cell gene transfer. *Nature Immunology* 2002; **3**: 318–321.
15. Aiuti A, Ficara F, Cattaneo F, et al. Gene therapy for adenosine deaminase deficiency. *Current Opinion in Allergy and Clinical Immunology* 2003; **3**: 461–466.
16. Kantoff P, Kohn D, Mitsuya H, et al. Correction of adenosine deaminase deficiency in cultured human T and B cells by retrovirus-mediated gene transfer. *Proceedings of National Academy Sciences, USA* 1986; **83**: 6563–6567.
17. Ferrari G, Rossini S, Giavazzi R, et al. An in vivo model of somatic cell gene therapy for human severe combined immunodeficiency. *Science* 1991; **251**: 1363–1366.
18. Ferrari G, Rossini S, Nobili N, et al. Transfer of the ADA gene into human ADA-deficient T lymphocytes reconstitutes specific immune functions. *Blood* 1992; **80**: 1120–1124.
19. Hirschhorn R, Yang DR, Puck JM, et al. Spontaneous in vivo reversion to normal of an inherited mutation in a patient with adenosine deaminase deficiency. *Nature Genetics* 1996; **13**: 290–295.
20. Arredondo-Vega FX, Santisteban I, Richard E, et al. Adenosine deaminase deficiency with mosaicism for a “second-site suppressor” of a splicing mutation: decline in revertant T lymphocytes during enzyme replacement therapy. *Blood* 2002; **99**: 1005–1013.
21. Blaese M, Anderson F, Culver K, et al. The ADA human gene therapy clinical protocol. *Human Gene Therapy* 1990; **1**: 327–362.
22. Bordignon C, Mavilio F, Ferrari G, et al. Transfer of the ADA gene into bone marrow cells and peripheral blood lymphocytes for the treatment of patients affected by ADA-deficient SCID. *Human Gene Therapy* 1993; **4**: 513–520.
- \* 23. Bordignon C, Notarangelo LD, Nobili N, et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA- immunodeficient patients. *Science* 1995; **270**: 470–475.
- \* 24. Blaese RM, Culver KW, Miller AD, et al. Lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science* 1995; **270**: 475–480.
- \* 25. Kohn DB, Weinberg KI, Nolte JA, et al. Engraftment of gene-modified umbilical cord blood cells in neonates with adenosine deaminase deficiency. *Nature Medicine* 1995; **1**: 1017–1023.
26. Onodera M, Ariga T, Kawamura N, et al. Successful peripheral T-lymphocyte-directed gene transfer for a patient with severe combined immune deficiency caused by adenosine deaminase deficiency. *Blood* 1998; **91**: 30–36.
27. Kawamura N, Ariga T, Ohtsu M, et al. In vivo kinetics of transduced cells in peripheral T cell-directed gene therapy: role of CD8<sup>+</sup> cells in improved immunological function in an adenosine deaminase (ADA)-SCID patient. *The Journal of Immunology* 1999; **163**: 2256–2261.
- \* 28. Aiuti A, Vai S, Mortellaro A, et al. Immune reconstitution in ADA-SCID after PBL gene therapy and discontinuation of enzyme replacement. *Nature Medicine* 2002; **8**: 423–425.

- \* 29. Muul LM, Tuschong LM, Soenen SL, et al. Persistence and expression of the adenosine deaminase gene for 12 years and immune reaction to gene transfer components: long-term results of the first clinical gene therapy trial. *Blood* 2003; **101**: 2563–2569.
30. Aiuti A. Advances in gene therapy for ADA-deficient SCID. *Current Opinion in Molecular Therapeutics* 2002; **4**: 515–522.
31. Hoogerbrugge PM, van Beusechem V, Fischer A, et al. Bone marrow gene transfer in three patients with adenosine deaminase deficiency. *Gene Therapy* 1996; **3**: 179–183.
- \* 32. Kohn DB, Hershfield MS, Carbonaro D, et al. Lymphocytes with a normal ADA gene accumulate after transplantation of transduced autologous umbilical cord blood CD34 + cells in ADA-deficient SCID neonates. *Nature Medicine* 1998; **4**: 775–780.
33. Schmidt M, Carbonaro DA, Speckmann C, et al. Clonality analysis after retroviral-mediated gene transfer to CD34 + cells from the cord blood of ADA-deficient SCID neonates. *Nature Medicine* 2003; **9**: 463–468.
- \* 34. Aiuti A, Slavin S, Aker M, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* 2002; **296**: 2410–2413.
35. Murray L, Luens K, Tushinski R, et al. Optimization of retroviral gene transduction of mobilized primitive hematopoietic progenitors by using thrombopoietin, Flt3, and Kit ligands and RetroNectin culture. *Human Gene Therapy* 1999; **10**: 1743–1752.
36. Glimm H & Eaves CJ. Direct evidence for multiple self-renewal divisions of human in vivo repopulating hematopoietic cells in short-term culture. *Blood* 1999; **94**: 2161–2168.
37. Abonour R, Williams DA, et al. Einhorn L. Efficient retrovirus-mediated transfer of the multidrug resistance 1 gene into autologous human long-term repopulating hematopoietic stem cells. *Nature Medicine* 2000; **6**: 652–658.
38. Dunbar CE, Takatoku M & Donahue RE. The impact of ex vivo cytokine stimulation on engraftment of primitive hematopoietic cells in a non-human primate model. *Annals of the New York Academy of Sciences* 2001; **938**: 236–244. discussion 244–235.
39. Dando JS, Aiuti A, Deola S, et al. Optimisation of retroviral supernatant production conditions for the genetic modification of human CD34 + cells. *The Journal of Gene Medicine* 2001; **3**: 219–227.
40. Williams DA. Retroviral-fibronectin interactions in transduction of mammalian cells. *Annals of the New York Academy of Sciences* 1999; **872**: 109–113. discussion 113–104.
41. Bolinger AM, Zangwill AB, Slattery JT, et al. An evaluation of engraftment, toxicity and busulfan concentration in children receiving bone marrow transplantation for leukemia or genetic disease. *Bone Marrow Transplant* 2000; **25**: 925–930.
42. Slavin S, Aker M, Shapira MY, et al. Non-myeloablative stem cell transplantation for the treatment of cancer and life-threatening non-malignant disorders; past accomplishments and future goals. *Transfusion & Apheresis Science* 2002; **27**: 159–166.
43. Huhn RD, Tisdale JF, Agricola B, et al. Retroviral marking and transplantation of rhesus hematopoietic cells by nonmyeloablative conditioning. *Human Gene Therapy* 1999; **10**: 1783–1790.
44. Rosenzweig M, MacVittie TJ, Harper D, et al. Efficient and durable gene marking of hematopoietic progenitor cells in nonhuman primates after nonablative conditioning. *Blood* 1999; **94**: 2271–2286.
45. Aiuti A, Cattaneo F, Cassani B, et al. Safety and efficacy of stem cell gene therapy combined with nonmyeloablative conditioning for the treatment of ADA-SCID. *Blood* 2003; **102**: 531.
46. Bonini C, Grez M, Traversari C, et al. Safety of retroviral gene marking with a truncated NGF receptor. *Nature Medicine* 2003; **9**: 367–369.
47. Kohn DB, Sadelain M, Dunbar C, et al. American Society of Gene Therapy (ASGT) Ad Hoc Subcommittee on retroviral-mediated gene transfer to hematopoietic stem cells. *Molecular Therapy* 2003; **8**: 180–187.
- \* 48. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003; **302**: 415–419.
49. Kohn DB, Sadelain M & Glorioso JC. Occurrence of leukaemia following gene therapy of X-linked SCID. *Nature Review Cancer* 2003; **3**: 477–488.
50. Berns A. Good news for gene therapy. *New England Journal of Medicine* 2004; **350**: 1679–1680.
51. Dave UP, Jenkins NA & Copeland NG. Gene therapy insertional mutagenesis insights. *Science* 2004; **303**: 333.
52. Cavazzana-Calvo M, Thrasher A & Mavilio F. The future of gene therapy. *Nature* 2004; **427**: 779–781.
53. Candotti F, Podsakoff G, Schurman S, et al. Corrective gene transfer into bone marrow CD34 + cells for adenosine deaminase (ADA) deficiency: results in four patients after one year of follow-up. *Molecular Therapy* 2003; **7**: S448–S449.

54. Cavazzana-Calvo M, Hacein BS, de Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000; **288**: 669–672.
- \* 55. Hacein-Bey-Abina S, Le Deist F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *New England Journal of Medicine* 2002; **346**: 1185–1193.
56. Fischer A, Landais P, Friedrich W, et al. European experience of bone-marrow transplantation for severe combined immunodeficiency. *Lancet* 1990; **336**: 850–854.
57. Haddad E, Landais P, Friedrich W, et al. Long-term immune reconstitution and outcome after HLA-identical T-cell-depleted bone marrow transplantation for severe combined immunodeficiency: a European retrospective study of 116 patients. *Blood* 1998; **91**: 3646–3653.