

# Hematopoietic stem cell gene therapy for adenosine deaminase deficient-SCID

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**Abstract** Gene therapy is a highly attractive strategy for many types of inherited disorders of the immune system. Adenosine deaminase (ADA) deficient-severe combined immunodeficiency (SCID) has been the target of several clinical trials based on the use of hematopoietic stem/progenitor cells engineered with retroviral vectors. The introduction of a low intensity conditioning regimen has been a crucial factor in achieving stable engraftment of hematopoietic stem cells and therapeutic levels of ADA-expressing cells. Recent studies have demonstrated that gene therapy for ADA-SCID has favorable safety profile and is effective in restoring normal purine metabolism and immune functions. Stem cell gene therapy combined with appropriate conditioning regimens might be extended to other genetic disorders of the hematopoietic system.

**Keywords** Gene therapy · Hematopoietic stem cell · Bone marrow · Severe combined immunodeficiency · Retroviral vector

## Introduction

Somatic gene therapy is a promising therapeutic option for genetic disorders of the immune system [1, 2]. This approach is based on the principle that a genetic defect can be amended by stably introducing a healthy copy of the defective gene in the target cell population or its progenitor cells. RNA viral vectors derived from murine gammaretroviruses have been

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used in clinical trials since 1990 to deliver therapeutic genes for the treatment of severe combined immunodeficiencies (SCID) [2, 3]. Adenosine deaminase (ADA) deficient-SCID has been the target of intensive preclinical and clinical investigation and represents a paradigmatic model of gene therapy for inherited disorders [4, 5].

### **ADA deficient-SCID: disease characteristics and current therapeutic strategies**

ADA-deficiency is the cause of approximately 15–20% of all cases of SCID, with an overall prevalence ranging from 1:375,000 to 1:660,000 live births. The ADA enzyme catalyses the irreversible deamination of adenosine (Ado) and deoxyadenosine (dAdo) in the purine catabolic pathway. The disease is characterized by impaired T, B, NK cell development and function, recurrent infections, and failure to thrive. In addition, non-immunological abnormalities occur in several organs, including the liver, lung, skeleton, and brain, as the consequence of the systemic metabolic defect due to the accumulation of purine toxic metabolites [6, 7].

Bone marrow transplant from an HLA-identical sibling donor is the treatment of choice for ADA-SCID, but available for a minority of patients. Despite recent improvements in the outcome of alternative transplants, the use of alternative bone marrow donor is still associated with high morbidity and mortality [8]. Indeed, the survival after transplant of hematopoietic stem cells (HSC) from matched unrelated donors (MUD) or mismatched related donors (MMRD) is reported in European registries to be 63% and 30%, respectively [8, 9]. The administration of enzyme replacement therapy (pegylated bovine ADA, PEG-ADA) results in clinical improvement with about 70–80% survival, but the immunological reconstitution is often incomplete [7, 8]. In addition, its high costs represent a major drawback for its worldwide diffusion and a considerable burden for national health systems.

### **Rationale for gene therapy and comparison with other treatments**

Several lines of evidence support the use of autologous gene corrected cells for ADA deficient-SCID [6]. First, the ADA gene is a housekeeping gene, expressed in all body cells. Therefore, even poorly regulated promoters such as the one present in gammaretroviral vectors can be used. Second, enzymatic levels as low as 10% allow normal immune functions in healthy individuals carrying a normal ADA gene. Third, normal or gene corrected cells have a selective survival advantage over deficient cells in hematopoietic cell transplantation and preclinical gene therapy models [4]. Thus, even relatively low amounts of engrafted HSC may result in successful therapy.

Gene therapy has several potential advantages over existing treatment methods suitable for patients who do not have access to an HLA-identical sibling donor [3]. With respect to BMT, transplantation of transduced HSC is potentially applicable to all ADA-SCID patients, independent from the availability of a donor, since it is an autologous procedure and there is no delay for donor search. Moreover, the use of autologous gene corrected cells avoids rejection and graft-versus-host disease due to HLA-mismatches or minor antigens incompatibility. Finally, gene therapy does not require the use of immunosuppressive prophylaxes or high dose conditioning regimens associated with organ toxicity (liver, lung, kidney, CNS), prolonged period of myelosuppression, and increased risks of infections.

Gene therapy has also a great potential advantage over enzyme replacement therapy. Indeed, a single infusion of gene corrected HSC may be sufficient to treat a patient lifelong,

thus avoiding the need for continuous supplementation of the enzyme and its high burden in terms of costs and patients' quality of life. Moreover, there is evidence from preclinical studies and bone marrow transplant that endogenous ADA is more effective than exogenously administered ADA [6, 10].

On the other hand, gene therapy can present also some peculiar risks and limitations. Integrating vectors carry an inherent risk of insertional mutagenesis associated with retroviral insertions at sensitive genomic sites [11]. Vector regulatory elements promoters may "turn-on" cellular proto-oncogenes adjacent to where they integrate, which, in combination to disease-specific risk factors, may lead to the appearance of clonal expansion or leukemic proliferation, as occurred in the case of chronic granulomatous disease [12] and SCID-X1 [13, 14]. So far, the experience of gene therapy for ADA-SCID did not reveal the occurrence of such complications [15], indicating that it has a favorable risk-benefit profile. Being an autologous procedure, a low content in autologous bone marrow HSC or pre-existing chromosomal alterations in bone marrow progenitors, may exclude patients from gene therapy [16]. Finally, the efficiency of gene transfer and the level of transgene expression can represent important limiting factor for the successful outcome of the procedure.

### **Gene therapy studies with mature T lymphocytes**

Gene therapy pilot studies for ADA deficient-SCID using retroviral vectors started in 1991. They differed considerably in terms of target cells, transduction procedures, and clinical protocols (reviewed in [4]). The first approaches were based on the infusion of autologous mature T lymphocytes, transduced with non-competent retroviral vectors derived from Moloney Murine Leukemia Virus (MLV), carrying the ADA gene cDNA. Patients who were eligible for gene therapy were chosen among those lacking a matched familiar donor or displaying inadequate immune reconstitution after enzymatic replacement therapy. All the patients enrolled in pilot studies continued to receive PEG-ADA, in order to avoid the risk of a further deterioration of immune function, being the efficacy of gene therapy still under investigation. Seven ADA-SCID patients have been reported to be treated with multiple infusions of gene corrected PBL, in three different clinical trials [17–20]. No adverse events or toxicity have been observed, and in the majority of patients and transduced T cells persisted many years after infusions were discontinued, demonstrating the safety and the therapeutic potential of PBL gene therapy. However, the clinical efficacy has been difficult to evaluate because all patients continued to receive enzyme replacement therapy. In one patient, in whom PEG-ADA was discontinued, gene corrected T cells progressively took the place of the ADA-deficient cells, resulting in improvement of T-cell counts, restoration of normal T-cell functions, and antibody responses to a neoantigen. This study was instrumental in demonstrating that withdrawal of PEG-ADA favored a selective growth advantage for gene corrected cells. However, infusion of gene corrected T cells was not sufficient to allow full correction of the metabolic defect [21], indicating the need for a broader reconstitution of ADA expression in multiple lineages.

### **Pilot gene therapy trials with hematopoietic stem cells**

Autologous HSC have been considered the optimal target cells for definitive correction of the metabolic and immunological defects in ADA-SCID patients. The first two clinical trials were based on transduction and reinfusion of bone marrow or cord blood progenitors cells

**Table 1** Summary of patients enrolled in clinical trials of gene transfer for ADA-SCID involving hematopoietic stem/progenitor cells. BM, bone marrow, UCB, umbilical cord blood

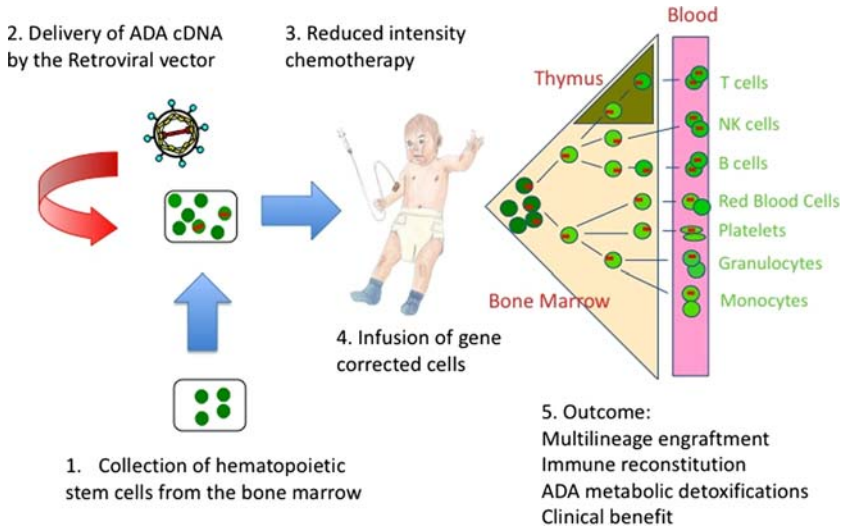
Investigators	Number of patients	Target cell	PEG-ADA after gene therapy	Conditioning
Bordignon et al. [18]	2	BM mononuclear cells	Yes	No
Kohn et al. [22, 23]	3	UCB CD34 <sup>+</sup> cells	Yes, briefly discontinued in one patient	No
Aiuti et al. [33, 35]	10	BM CD34 <sup>+</sup> cells	No	Busulfan (4 mg/Kg)
Sokolic et al. [39]	4	BM CD34 <sup>+</sup> cells	Yes	No
Sokolic et al. [39]	4	BM CD34 <sup>+</sup> cells	No	Busulfan (75 mg/m <sup>2</sup> )
Engel et al. [16]				
Gaspar et al. [37]	1	BM CD34 <sup>+</sup> cells	No	Melphalan (140 mg/m <sup>2</sup> )
Otsu et al. [38]	2	BM CD34 <sup>+</sup> cells	No	No

transduced with a retroviral vector encoding ADA, without any preparative conditioning [18, 22, 23] (Table 1). In the clinical trial conducted at the Children's Hospital of Los Angeles in three ADA-SCID patients, lymphocytes derived from ADA-transduced cord blood HSC accumulated over time and were detectable for at least 8 years after the procedure [24], indicating a selective advantage for the T-cell lineage. The study performed at the San Raffaele Scientific Institute in Milano showed that ex vivo transduced bone marrow progenitor cells were capable of differentiating in vivo into multiple lineages, but did not achieve therapeutic levels of ADA expression [18]. Overall, both studies were limited by a low gene transfer efficiency and engraftment level of pluripotent HSC, which did not result in significant clinical benefit. In addition, immune functions were not proven to be sustained by gene corrected cells, since all patients continued to receive enzyme replacement therapy.

### The HSR-TIGET clinical trial: introducing nonmyeloablative conditioning in gene therapy

The cumulative experience of the early gene therapy trials pointed out the need to improve gene transfer efficiency and favor the engraftment of long-lasting gene corrected HSC, in order to increase the pool of transduced progenitors and extend the correction of the ADA defect to all hematopoietic lineages. The experience in SCID patients who received bone marrow transplantation without cytoreductive regimen taught us that donor T lymphocyte lineages engraft long-term while most or all of the B lymphoid and myeloid lineage cells remain those of the host [25]. In contrast, the use of chemotherapeutic conditioning of the recipient permits the engraftment of HSC and all lympho-hematopoietic lineages.

In 2000 we started the first clinical trial of gene therapy administering a chemotherapeutic agent to 'make space' in the bone marrow, in order to increase the engraftment of gene corrected HSC (Fig. 1). The rationale for the adoption of a nonmyeloablative regimen originated from gene marking studies in animal models and from the transplantation



**Fig. 1** Gene therapy with hematopoietic stem cells. Schematic representation of the main phases of the gene therapy clinical protocol for ADA-SCID conducted at HSR-TIGET. Patients received the infusion of autologous gene corrected bone marrow CD34<sup>+</sup> cells combined to low dose busulfan, resulting in correction of the metabolic and immunological defect and clinical benefit

experiences in the field of hematological pediatric disorders [26–28]. In particular, busulfan, used alone, was chosen because of its potent effects on primitive hematopoietic progenitors [29], and the lack of organ toxicity at reduced doses in nonmyeloablative regimens for HSC transplant, including pediatric SCID patients [30]. The administered dose (2 mg/Kg for 2 consecutive days) represented about 25% of the total dose of busulfan used in fully myeloablative protocols. In addition, the transduction protocol was optimized by the use of a retroviral supernatant produced in conditions that were adapted for human CD34<sup>+</sup> cells [31], and target cells were transduced in the presence of retronectin [32] and of a culture medium containing an optimized cytokine cocktail, including FLT3-ligand, stem cell factor, thrombopoietin, and IL-3 [33, 34].

Two patients, who lacked an HLA-identical sibling donor and did not have access to PEG-ADA therapy, were enrolled in the pilot clinical trials carried out at the Hadassah University Hospital (Jerusalem) and at HSR-TIGET (Milano), respectively [33] (Table 1). Gene therapy resulted in an engraftment that was sustained by transduced CD34<sup>+</sup> BM progenitor cells, with multilineage differentiation, increase of lymphocyte counts, improvement of cellular and humoral responses, and a substantial decrease in toxic metabolites. These results represented the first demonstration of the clinical efficacy of HSC gene therapy for ADA-SCID. Subsequently, eight additional children affected by early onset of SCID were enrolled and treated according to the same strategy (Table 1) [35]. Patients lacked a compatible sibling donor and PEG-ADA were unavailable or did not achieve adequate immune reconstitution. In the latter case, enzyme replacement therapy was discontinued to fully exploit the selective growth advantage for gene corrected over defective cells and to evaluate the efficacy of gene therapy alone.

At present, all the 10 treated patients are alive and in good clinical conditions, with the exception of one patient who requires steroid treatment due to recurrent episodes of autoimmunity, previously observed while on PEG-ADA [35]. Nine of the ten ADA-SCID

patients treated displayed progressive restoration of immune functions, improvement of patients' physical development, and protection from severe infections. None of the patients showed clonal proliferation or adverse events related to gene transfer, with a median follow-up of 4 years [35]. All children maintain stable engraftment of vector ADA-transduced cells with sustained ADA enzymatic activity and efficient systemic detoxification, with the longest follow at 8 years after treatment. Gene corrected cells were detected in all myeloid and lymphoid subsets, the latter being more represented due to their survival advantage. Clonal analysis of long-term repopulating cell progeny revealed polyclonal T-cell populations, with >100 different gene corrected progenitors contributing to T lymphopoiesis [15]. In addition, the presence of shared vector integrations among multiple hematopoietic lineages demonstrated the engraftment of multipotent HSC [15]. Gene therapy led to a recovery of thymic activity, significant increase in lymphocyte counts and normalization of T-cell functions, including sensitivity to apoptosis, cytokine production, and proliferative responses upon mitogens and antigens stimulation [36]. Serum Ig levels increased, and IVIG were discontinued in five patients with evidence of antigen-specific antibodies to vaccinal antigens and pathogens [35]. In summary, these results show the clinical efficacy of ADA gene transfer in restoring normal immune and metabolic functions of ADA-SCID patients.

### Other gene therapy trials for ADA-SCID

A group at Great Ormond Street Hospital (London) has reported the success of a gene therapy clinical trial for ADA-deficient SCID in a patient with an inadequate response to PEG-ADA [37]. As conditioning regimen, a single dose of melphalan (140 mg/m<sup>2</sup>) was used instead of busulfan. Following reinfusion of transduced bone marrow cells, the majority of T cells and NK cells were gene corrected, but a lower proportion of vector positive B cells (10%) and granulocytes (0.1%) were observed, as compared to the HSR-TIGET clinical trial, indicating that type of cytoreductive agents may influence differently HSC engraftment. Two years after the procedure thymopoiesis had recovered after the procedure and improvement in T-cell counts was observed. ADA expression was observed in different lineages, including red blood cells, and adequate metabolic detoxification was detected in the absence of external detoxification, with no adverse events related to gene transfer.

Another group in Japan reported at the 2006 meeting of the American Society of Gene Therapy (ASGT) the first results of a different clinical trial based on infusion of autologous bone marrow CD34<sup>+</sup> cells in two ADA-SCID children (Table 1). In this case, PEG-ADA was discontinued to enhance selective advantage, but no cytoreductive preparative regimen was given. The investigators reported a delayed immune reconstitution and effective detoxification of purine metabolites, although no information on engraftment of HSC was available [38].

The groups at the National Institute of Health and Children's Hospital of Los Angeles recently reported at the 2008 ASGT meeting the preliminary outcome of two clinical trials of gene transfer in bone marrow CD34<sup>+</sup> cells, designed originally to compare the efficiency of two different retroviral vectors [39] (Table 1). In the first protocol, four patients were treated with no myelosuppression, and continued to receive enzyme replacement therapy. Low levels of marking were detected in two patients (<1%) and no sustained improvement in immunological parameters were observed during the follow-up. In the second clinical trial, the investigators adopted a design similar to the HSR-TIGET

protocol, based on busulfan administration and withdrawal of PEG-ADA. The preliminary data indicate that this approach leads to improved immunological and metabolic outcome as compared to the first version of the clinical trial [39]. Another patient experienced a prolonged cytopenia following busulfan conditioning, which was related to a pre-existing cytogenetic abnormality, indicating an important limitation to be considered for autologous HSC gene transfer [16].

### **Safety of gene therapy for ADA-SCID**

Gene therapy has been shown to be beneficial for patients affected by SCID-X1 and CGD, but its application was seriously limited by the occurrence of clonal expansion and leukemic proliferation associated with retroviral vector integrations in the proximity of cellular proto-oncogenes [12–14]. Since 1990, over 35 ADA-SCID patients have been enrolled in phase I/II gene therapy trials in 10 different clinical trials [3, 5]. The cumulative experience of our study [5, 8] as well as of other clinical trials for ADA deficient-SCID [3] (Table 1), indicates that gene therapy for ADA deficient-SCID has a favorable risk-benefit profile, since no event of insertional oncogenesis have been observed over a long period of follow-up. This is consistent with the polyclonal pattern of vector integration and T-cell repertoire [15], the lack of *in vivo* skewing for potentially dangerous insertions [35], and the absence of transcriptional perturbation of cellular proto-oncogenes (Cassani and Aiuti, unpublished results). Unique factors typical of the SCID-X1 or CGD trials may have contributed to the different outcomes, including specific vector promoter and constructs, inappropriate expression of transgene products involved in cell signaling [3], cooperation between transgene and cellular proto-oncogenes [40], or disease background [41] associated with high rate of transformation. The use of self-inactivating vectors with improved safety profile, such as lentiviral vectors [42, 43], might increase the safety of gene therapy for diseases carrying an intrinsic higher risk due to the disease or transgene [44, 45].

### **Perspectives for other diseases**

Our study suggests that gene transfer into HSC combined with appropriate conditioning regimens could be successfully extended to other inherited blood borne disorders. Indeed, the average level of 5% correction in bone marrow progenitors observed in our trial is clinically relevant for an inherited disorder with a relatively low therapeutic threshold, such as certain metabolic disorders, or for other primary immunodeficiencies in which transduced lymphocytes can expand preferentially due to a selective survival or proliferative advantage, such as Wiskott-Aldrich Syndrome [46, 47]. On the other hand, lysosomal storage disorders or thalassemia will require the use of more efficient and regulated vectors such as lentiviral vectors [42], as well as of more intense preparative regimens to enhance the survival advantage of gene modified HSC.

### **Conclusions**

The gene transfer trials for ADA-SCID represent a paradigmatic approach for gene therapy of inherited disorders of the immune system. The pilot studies showed the therapeutic potential and limitations of gene transfer into HSC. The introduction of improved gene

transfer protocol, the use of a reduced intensity conditioning, and withdrawal of enzyme replacement therapy were instrumental in the successful outcome of recent clinical trials, allowing to achieve full correction of the immune and metabolic defects, with proven clinical benefit. No adverse events related to gene transfer were observed, but safety monitoring will be continued to be implemented long-term in all patients. Taken together, the results of the most recent gene therapy studies indicate that gene therapy with non-myeloablative conditioning is now an option to be considered for all ADA-SCID patients lacking an HLA-identical sibling donor.

## References

1. Bordignon C, Roncarolo MG. Therapeutic applications for hematopoietic stem cell gene transfer. *Nat Immunol.* 2002;3:318–21.
2. Fischer A, Le Deist F, Hacein-Bey-Abina S, Andre-Schmutz I, Basile Gde S, de Villartay JP, et al. Severe combined immunodeficiency. A model disease for molecular immunology and therapy. *Immunol Rev.* 2005;203:98–109.
3. Kohn DB. Gene therapy for childhood immunological diseases. *Bone Marrow Transplant.* 2008;41:199–205.
4. Aiuti A, Ficara F, Cattaneo F, Bordignon C, Roncarolo MG. Gene therapy for adenosine deaminase deficiency. *Curr Opin Allergy Clin Immunol.* 2003;3:461–6.
5. Aiuti A, Bachoud-Levi AC, Blesch A, Brenner MK, Cattaneo F, Chiocchia EA, et al. Progress and prospects: gene therapy clinical trials (part 2). *Gene Ther.* 2007;14:1555–63.
6. Hirschorn R, Candotti F. Immunodeficiency due to defects of purine metabolism. In: Ochs H, Smith C, Puck J, editors. *Primary immunodeficiency diseases.* Oxford: Oxford University Press; 2006. p. 169–96.
7. Hershfield MS. Adenosine deaminase deficiency: clinical expression, molecular basis, and therapy. *Semin Hematol.* 1998;35:291–8.
8. Booth C, Hershfield M, Notarangelo L, Buckley R, Hoenig M, Mahlaoui N, et al. Management options for adenosine deaminase deficiency: proceedings of the EBMT satellite workshop (Hamburg, March 2006). *Clin Immunol.* 2007;123:139–47.
9. Honig M, Albert MH, Schulz A, Sparber-Sauer M, Schutz C, Belohradsky B, et al. Patients with adenosine deaminase deficiency surviving after hematopoietic stem cell transplantation are at high risk of CNS complications. *Blood.* 2006;109:3595–602.
10. Ferrari G, Rossini S, Giavazzi R, Maggioni D, Nobili N, Soldati M, et al. An in vivo model of somatic cell gene therapy for human severe combined immunodeficiency. *Science.* 1991;251:1363–6.
11. Baum C, Kustikova O, Modlich U, Li Z, Fehse B. Mutagenesis and oncogenesis by chromosomal insertion of gene transfer vectors. *Hum Gene Ther.* 2006;17:253–63.
12. Ott MG, Schmidt M, Schwarzwaelder K, Stein S, Siler U, Koehl U, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVII1, PRDM16 or SETBP1. *Nat Med.* 2006;12:401–9.
13. Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest.* 2008;118:3132–42.
14. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempinski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest.* 2008;118:3143–50.
15. Aiuti A, Cassani B, Andolfi G, Mirolo M, Biasco L, Recchia A, et al. Multilineage hematopoietic reconstitution without clonal selection in ADA-SCID patients treated with stem cell gene therapy. *J Clin Invest.* 2007;117:2233–40.
16. Engel BC, Podsakoff GM, Ireland JL, Smogorzewska EM, Carbonaro DA, Wilson K, et al. Prolonged pancytopenia in a gene therapy patient with ADA-deficient SCID and trisomy 8 mosaicism: a case report. *Blood.* 2007;109:503–6.
17. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, et al. T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science.* 1995;270:475–80.
18. Bordignon C, Notarangelo LD, Nobili N, Ferrari G, Casorati G, Panina P, et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. *Science.* 1995;270:470–5.



19. Onodera M, Ariga T, Kawamura N, Kobayashi I, Ohtsu M, Yamada M, et al. Successful peripheral T-lymphocyte-directed gene transfer for a patient with severe combined immune deficiency caused by adenosine deaminase deficiency. *Blood*. 1998;91:30–6.
20. Muul LM, Tuschong LM, Soenen SL, Jagadeesh GJ, Ramsey WJ, Long Z, 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;101:2563–9.
21. Aiuti A, Vai S, Mortellaro A, Casorati G, Ficara F, Andolfi G, et al. Immune reconstitution in ADA-SCID after PBL gene therapy and discontinuation of enzyme replacement. *Nat Med*. 2002;8:423–5.
22. Kohn DB, Hershfield MS, Carbonaro D, Shigeoka A, Brooks J, Smogorzewska EM, et al. T lymphocytes with a normal ADA gene accumulate after transplantation of transduced autologous umbilical cord blood CD34+ cells in ADA-deficient SCID neonates. *Nat Med*. 1998;4:775–80.
23. Kohn DB, Weinberg KI, Nolta JA, Heiss LN, Lenarsky C, Crooks GM, et al. Engraftment of gene-modified umbilical cord blood cells in neonates with adenosine deaminase deficiency. *Nat Med*. 1995;1:1017–23.
24. Schmidt M, Carbonaro DA, Speckmann C, Wissler M, Bohnsack J, Elder M, et al. Clonality analysis after retroviral-mediated gene transfer to CD34+ cells from the cord blood of ADA-deficient SCID neonates. *Nat Med*. 2003;9:463–8.
25. Haddad E, Landais P, Friedrich W, Gerritsen B, Cavazzana CM, Morgan G, et al. Long-term immune reconstitution and outcome after HLA-nonidentical T-cell-depleted bone marrow transplantation for severe combined immunodeficiency: a European retrospective study of 116 patients. *Blood*. 1998;91:3646–53.
26. Huhn RD, Tisdale JF, Agricola B, Metzger ME, Donahue RE, Dunbar CE. Retroviral marking and transplantation of rhesus hematopoietic cells by nonmyeloablative conditioning. *Hum Gene Ther*. 1999;10:1783–90.
27. Rosenzweig M, MacVittie TJ, Harper D, Hempel D, Glickman RL, Johnson RP, et al. Efficient and durable gene marking of hematopoietic progenitor cells in nonhuman primates after nonablative conditioning. *Blood*. 1999;94:2271–86.
28. Slavin S, Aker M, Shapira MY, Panigrahi S, Gabriel C, Or R. Non-myeloablative stem cell transplantation for the treatment of cancer and life-threatening non-malignant disorders; past accomplishments and future goals. *Transfus Apher Sci*. 2002;27:159–66.
29. Kuramoto K, Follman D, Hematti P, Sellers S, Laukkanen MO, Seggewiss R, et al. The impact of low-dose busulfan on clonal dynamics in nonhuman primates. *Blood*. 2004;104:1273–80.
30. Bolinger AM, Zangwill AB, Slaterry JT, Glidden D, DeSantes K, Heyn L, 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;25:925–30.
31. Dando JS, Aiuti A, Deola S, Ficara F, Bordignon C. Optimisation of retroviral supernatant production conditions for the genetic modification of human CD34+ cells. *J Gene Med*. 2001;3:219–27.
32. Williams DA. Retroviral-fibronectin interactions in transduction of mammalian cells. *Ann N Y Acad Sci*. 1999;872:109–13. discussion 113–104.
33. Aiuti A, Slavin S, Aker M, Ficara F, Deola S, Mortellaro A, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science*. 2002;296:2410–3.
34. Ficara F, Superchi DB, Hernandez RJ, Mocchetti C, Carballido-Perrig N, Andolfi G, et al. IL-3 or IL-7 increases ex vivo gene transfer efficiency in ADA-SCID BM CD34+ cells while maintaining in vivo lymphoid potential. *Mol Ther*. 2004;10:1096–108.
35. Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L, et al. Long-term safety and efficacy of gene therapy for adenosine deaminase (ADA)-deficient severe combined immunodeficiency. *New Engl J Med*. 2009;360:447–58.
36. Cassani B, Mirolo M, Cattaneo F, Benninghoff U, Hershfield M, Carlucci F, et al. Altered intracellular and extracellular signaling leads to impaired T-cell functions in ADA-SCID patients. *Blood*. 2008;111:4209–19.
37. Gaspar HB, Bjorkegren E, Parsley K, Gilmour KC, King D, Sinclair J, et al. Successful reconstitution of immunity in ADA-SCID by stem cell gene therapy following cessation of PEG-ADA and use of mild preconditioning. *Mol Ther*. 2006;14:505–13.
38. Otsu M, Nakajima M, Kida M, Maeyama Y, Toita N, Hatano N, et al. Stem cell gene therapy with no pre-conditioning for the ADA-deficiency patients leads to generalized detoxification and delayed, but steady hematological reconstitution. *Mol Ther*. 2006;13:S418. (Abstract).
39. Sokolic R, Podsakoff G, Muul L, Engel B, Jagadeesh J, Garabedian E, et al. Myelosuppression and withdrawal of PEG-ADA lead to superior results after gene therapy for adenosine deaminase deficiency (ADA-SCID). *Mol Ther*. 2008;16:S111. (Abstract).

40. Dave UP, Jenkins NA, Copeland NG. Gene therapy insertional mutagenesis insights. *Science*. 2004;303:333.
41. Shou Y, Ma Z, Lu T, Sorrentino BP. Unique risk factors for insertional mutagenesis in a mouse model of XSCID gene therapy. *Proc Natl Acad Sci USA*. 2006;103:11730–5.
42. Follenzi A, Ailles LE, Bakovic S, Geuna M, Naldini L. Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. *Nat Genet*. 2000;25:217–22.
43. Mortellaro A, Hernandez RJ, Guerrini MM, Carlucci F, Tabucchi A, Ponzoni M, et al. Ex vivo gene therapy with lentiviral vectors rescues adenosine deaminase (ADA)-deficient mice and corrects their immune and metabolic defects. *Blood*. 2006;108:2979–88.
44. De Palma M, Montini E, de Sio FR, Benedicenti F, Gentile A, Medico E, et al. Promoter trapping reveals significant differences in integration site selection between MLV and HIV vectors in primary hematopoietic cells. *Blood*. 2005;105:2307–15.
45. Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, et al. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. *Nat Biotechnol*. 2006;24:687–96.
46. Galy A, Roncarolo MG, Thrasher AJ. Development of lentiviral gene therapy for Wiskott Aldrich syndrome. *Expert Opin Biol Ther*. 2008;8:181–90.
47. Dupré L, Marangoni F, Scaramuzza S, Trifari S, Hernandez RJ, Aiuti A, et al. Efficacy of gene therapy for Wiskott-Aldrich syndrome using a WAS promoter/cDNA-containing lentiviral vector and nonlethal irradiation. *Hum Gene Ther*. 2006;17:303–13.