

New insights into the pathogenesis of adenosine deaminase-severe combined immunodeficiency and progress in gene therapy

Aisha V. Sauer^{a,b} and Alessandro Aiuti^{a,c}

^aSan Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), ^bUniversità Vita-Salute San Raffaele, Milan and ^cDepartment of Public Health and Cell Biology, Tor Vergata University, Rome, Italy

Correspondence to A. Aiuti, HSR-TIGET, Via Olgettina 58, 20132 Milano, Italy
Tel: +39 0226434435; fax: +39 0226434668;
e-mail: a.aiuti@hsr.it

Current Opinion in Allergy and Clinical Immunology 2009, 9:000–000

Purpose of review

Adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID) is a complex metabolic and immunological disorder, characterized by a severe immunodeficiency due to the accumulation of purine metabolites in plasma and cells. This review summarizes recent findings on the pathogenesis of immunological and nonimmunological defects in ADA deficiency and the successful outcome of gene therapy trials for this condition.

Recent findings

Recent reports show that ADA-SCID is associated with an increased frequency of autoimmune manifestations and high risk of central nervous system (CNS) complications even after bone marrow transplantation. It remains unclear to what extent infection-related or disease-specific factors correlate with this divergent outcome. Recent trials represented the first demonstration of long-term clinical efficacy of HSC gene therapy for ADA-SCID, underlining that gene therapy has a favorable safety profile and is effective in restoring normal purine metabolism and immune functions. Molecular studies showed that the retroviral integration profile after successful gene therapy did not cause selection or expansion of malignant cell clones *in vivo*.

Summary

Gene therapy for ADA-deficient SCID is an effective treatment, providing long-term clinical benefit for affected patients. Future research will be needed to address the occurrence of autoimmune manifestations and nonimmunological defects in order to improve patients' long-term prospects.

Keywords

adenosine deaminase, adenosine deaminase-severe combined immunodeficiency, gene therapy, severe combined immunodeficiency

Curr Opin Allergy Clin Immunol 9:000–000
© 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins
1528-4050

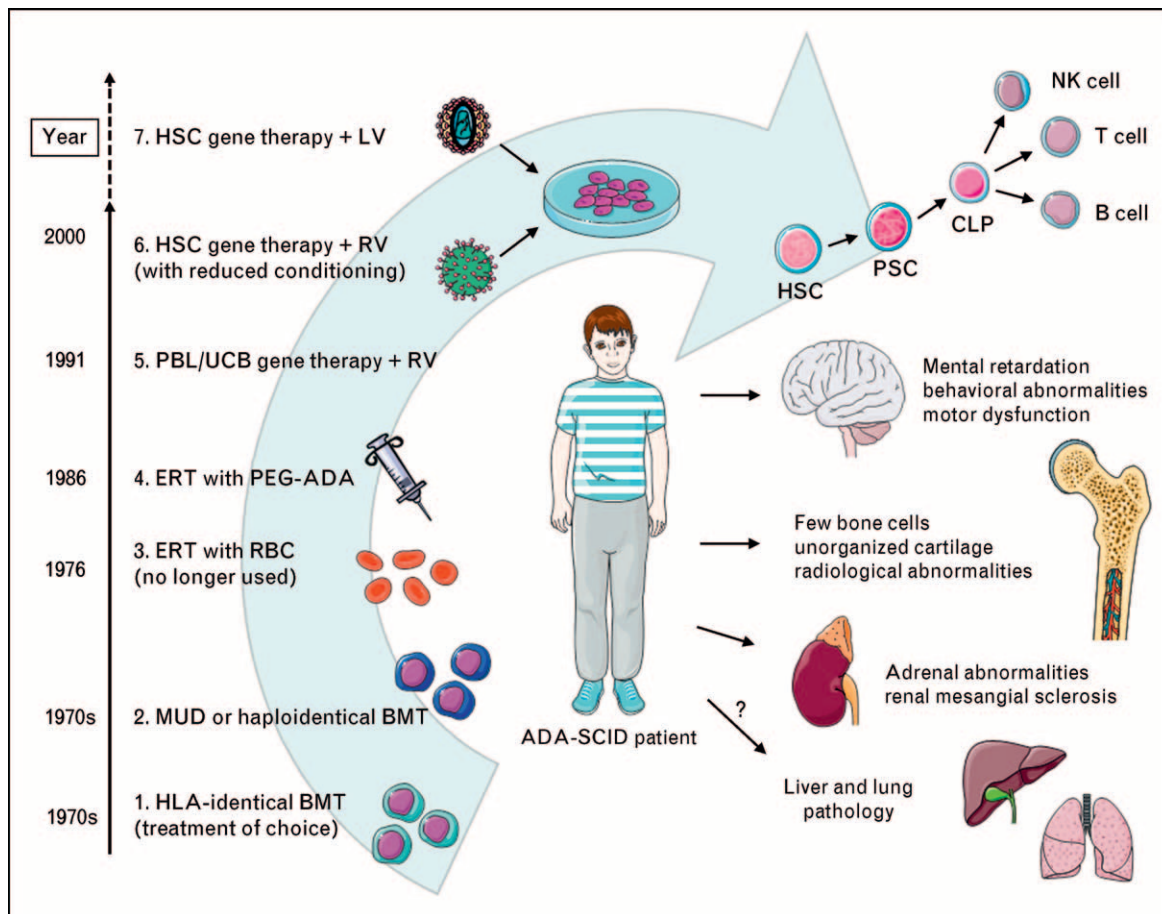
Introduction

ADA deficiency is the cause of approximately 15–20% of SCIDs, with an overall prevalence ranging from 1:375 000 to 1:660 000 live births. ADA catalyzes the irreversible deamination of adenosine and deoxyadenosine in the purine salvage pathway. The observed genotype and both the clinical and metabolic phenotype are strongly correlated. Mutations that express residual ADA activity have been retrieved from patients with milder forms or delayed/late onset phenotypes, whereas mutations retrieved in patients with SCID, the most common early-onset phenotype, are associated with very low or undetectable enzymatic activity [1]. Lack of ADA causes accumulation of purine metabolites leading to lymphopenia, absent cellular and humoral immunity, failure to thrive, and recurrent infection [1,2]. The additional presence of skeletal, hepatic, renal, lung,

and neurological abnormalities underlines the complexity of this multiorgan pathology and the systemic impact of the ADA metabolism.

In ADA-SCID patients, bone marrow transplantation (BMT) from an HLA-identical sibling donor is the treatment of choice, but available only for a minority of patients [3]. The administration of enzyme replacement therapy (ERT) with pegylated bovine ADA (PEG-ADA) results in clinical improvement with about 70–80% survival, but often incomplete immunological reconstitution. Anti-ADA antibodies can be detected in most patients, whereas neutralizing antibodies develop in about 10% of patients under ERT [1,4].

A strong scientific rationale together with the necessity for alternative treatment options made ADA-SCID the first candidate disease treated with gene therapy

Figure 1 Past, present and future development of therapeutic options in ADA-SCID and its immune and nonimmune manifestations

Therapeutic options for ADA-SCID patients comprise bone marrow transplantation, enzyme replacement or gene therapy, but recovery of immune functions may vary depending on the applied treatment and patient's characteristics. Treatment of choice remains bone marrow transplantation from an HLA-identical sibling donor, whereas transplants from alternative donors are associated with high morbidity and mortality. Enzyme replacement therapy, using partial exchange transfusion with normal, irradiated erythrocytes, is no longer used and has been substituted by the development of pegylated bovine ADA. Early gene therapy studies for ADA-SCID utilized peripheral blood lymphocytes or CD34+ cells isolated from patient's umbilical cord blood. Subsequent clinical trials moved to transduction and reinfusion of bone marrow cells. Until now all patients were treated with retroviral vectors containing the ADA cDNA. The use of self-inactivating (SIN) lentiviral vectors might provide a superior integration and safety profile for ADA-gene therapy in the future. Several nonimmune abnormalities have been described in ADA deficiency, indicating that the disease should be considered a 'systemic' metabolic disorder. These include hepatic, lung and renal disease, skeletal and bone growth abnormalities as well as neurological and behavioral impairments. BMT, bone marrow transplantation; CLP, committed lymphocyte precursor; ERT, enzyme replacement therapy; HLA, human leukocyte antigen; HSC, hematopoietic stem cell; LV, lentiviral vectors; MUD, matched unrelated donor; NK, natural killer cell; PBL, Peripheral blood lymphocytes; PEG-ADA, pegylated bovine ADA; PSC, pluripotent stem cell; RBC, red blood cells; RV, retroviral vectors; UCB, umbilical cord blood; (Original Figure, produced using Servier Medical Art).

worldwide. In the past decades gene therapy treatment in ADA deficient patients has evolved significantly and nowadays ADA deficient SCID is among the few diseases successfully treated with gene therapy [5*]. Since 1990, over 35 ADA-SCID patients have been enrolled in 10 different phase I/II clinical gene therapy trials.

Here we will review the most recent findings on the pathology and gene therapy treatment for ADA-deficient SCID. Figure 1 summarizes the development of therapeutic approaches for this disease and its immune and nonimmune manifestations.

Immunological defects and autoimmune manifestations in adenosine deaminase deficiency

Immunological defects associated with ADA-deficiency include impaired T, B, NK cell development and function, complete absence of cellular and humoral immunity and recurrent infections. It is well established that the metabolic basis underlying immune cell deficiency is related to the physiological impact of the ADA substrates, adenosine and deoxyadenosine. Deoxyadenosine behaves as a cytotoxic metabolite and is considered

the primary cause of lymphotoxicity in ADA-SCID, whereas adenosine may function as an extracellular signal transducer mediating a variety of physiological effects via adenosine receptor signaling.

Recently, the biochemical pathways responsible for the pathogenesis of the disease were investigated in CD4⁺ T-cell lines established from ADA-SCID patients [6[•]]. Cassani *et al.* [6[•]] reported that a TCR-dependent activation defect induced by residual intracellular toxicity and an active immunosuppression mediated by extracellular adenosine receptor triggering are responsible for the functional impairments and susceptibility to apoptosis of ADA-deficient T cells.

A key finding of milder forms of the disease or patients on PEG-ADA are manifestations of immune dysregulation including autoimmunity (type I diabetes, hypothyroidism, autoimmune thrombocytopenia, hemolytic anemia) and allergic manifestations [7,8]. Recently, autoimmune manifestations have also been reported in bone marrow transplanted patients [9[•],10[•]] as well as in patients treated with HSC gene therapy [11^{••}]. Little is known about the pathogenesis of these manifestations and whether their occurrence is related to alterations in central or peripheral tolerance. Particularly, it remains unclear to what extent some mechanisms of tolerance might be specifically affected in ADA deficiency.

It is important to note that extracellular adenosine produced by regulatory T cells, has been proposed as one of the mechanisms mediating their suppressive activity (reviewed in [12]). Regulatory T cells express CD39 and CD73 ectonucleotidases, which generate adenosine pericellularly in order to suppress the proliferation of T effector cells via the adenosine 2A receptor. Consequently, the autoimmune manifestations associated with ADA-deficiency might be the result of an altered purine metabolism interfering with normal regulatory T cell function. In order to prevent autoimmune manifestations in these patients and to adjust current treatment regimens, the contribution of both T-cell and/or B-cell deficiency and the ADA metabolism to immune dysregulation remains to be assessed.

Nonimmunological defects in adenosine deaminase deficiency

ADA is ubiquitously expressed in all cell types; when absent, the systemic metabolic toxicity is frequently associated with organ damage and neurological alterations. Several nonimmune abnormalities have been described in ADA deficiency, indicating that this disease should be considered a 'systemic' metabolic disorder [1,13]. These include hepatic and renal disease [14], skeletal alterations [1,15], neurological abnormalities

[1] and behavioral impairments [16]. Because complications from infections usually predominate in the clinical presentation of infants with ADA deficiency, the full spectrum of nonimmunologic manifestations of the disorder and their natural course may be obscured [17].

Two recent reports show that ADA-SCID patients after BMT are at high risk of CNS complications. Hönig *et al.* [17] analyzed the long-term outcome in 15 ADA-deficient patients treated with HSCT. Six of 12 surviving patients showed marked neurologic abnormalities, including mental retardation and motor dysfunction. A second, more comparative study assessed cognitive and behavioral function in 105 SCID patients after HSCT [18^{••}]. Multivariate analysis indicated that the underlying genetic defect, diagnosis of ADA-SCID, and consanguinity were associated with worse outcome but that age at transplantation and chemotherapy conditioning were not. In both studies the authors were unable to identify transplantation-related or disease-specific factors correlating with this divergent neurologic outcome. They, therefore, suggest metabolic abnormalities rather than complications of the treatment procedure or previous infections as determining factors leading to the observed neurological complications.

From detailed studies on the bone phenotype in ADA deficient mice and patients, we obtained evidence for specific skeletal defects related to ADA-SCID [15]. ADA deficiency in mice causes a specific bone phenotype characterized by alterations of structural properties and impaired mechanical competence, which is distinct from the phenotype observed in other immunodeficient mice. We extended our study to 15 ADA-SCID patients, displaying a similar misbalance in bone remodeling alongside severe growth retardation.

Although lung alterations do not represent a major phenotype of human ADA deficiency, it has been suggested that the purine metabolic disturbances may contribute to respiratory distress or asthma often observed in these patients. Recently, an ADA/adenosine 2B receptor (A2BR) double-knockout mouse model revealed a protective role for A2BR signaling in the lung pathology associated with ADA deficiency [19]. Contrarily to what was to be expected from pharmacological studies in ADA^{-/-} mice, demonstrating that A2BR antagonism can attenuate features of chronic lung disease, the double-knockout exhibited enhanced pulmonary inflammation and airway destruction, therefore supporting an important protective role for A2BR signaling during acute stages of lung disease [19].

Recent progress in gene therapy for adenosine deaminase deficiency

Early gene therapy studies for ADA-SCID began in 1990 using peripheral blood lymphocytes transduced with

retroviral vectors. Seven ADA-SCID patients enrolled in three different protocols were treated without any adverse events [20–22]. Gene corrected cells persisted for many years after infusions were discontinued. In parallel, clinical trials were carried out based on the use of bone marrow or cord blood progenitors cells which were administered without any preparative conditioning [21,23]. Nonetheless, due to low gene transfer efficiency and engraftment levels of HSC, no significant clinical benefit was achieved. In both cases immunological reconstitution was difficult to evaluate because all patients continued to receive PEG-ADA, which would have eliminated any selective advantage of the gene-corrected cells (reviewed in [13]).

The cumulative experience of the early gene therapy trials pointed out a need to improve gene transfer efficiency and favor the engraftment of long-lasting gene corrected HSC. Breakthrough came in 2000 with an improved transduction protocol of bone marrow CD34+ cells and the use of non-myeloablative chemotherapeutic conditioning to ‘make space’ in the bone marrow [2,24]. A total of 10 patients, who lacked an HLA-identical sibling donor, were enrolled in three phase I/II clinical protocols [2,11^{••}]. Gene therapy resulted in sustained engraftment of transduced CD34+ bone marrow progenitor cells, with multilineage differentiation, increase of lymphocyte counts and improvement of cellular and humoral responses [2]. PEG-ADA was discontinued to take advantage of the selective growth advantage for gene corrected over defective cells and to evaluate the efficacy of gene therapy alone. At present, all 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 [11^{••}]. Importantly, none of the patients showed clonal proliferation or adverse events related to gene transfer, with a median follow up of 4 years [11^{••}]. In the meantime five additional patients have been treated with encouraging results. These findings represented the first demonstration of the clinical efficacy of ADA gene transfer in restoring normal immune and metabolic functions of ADA-SCID patients.

Advantages of gene therapy

Gene therapy has several potential advantages over existing treatments for ADA deficiency [25[•]]. Independently from donor availability and without the risk of graft rejection and GvHD, autologous transduced HSC are potentially applicable to all ADA-SCID patients. With respect to ERT, a single infusion of gene corrected HSC may be sufficient to treat a patient lifelong, thus avoiding the need for weekly enzyme supplementation and its related burden in terms of costs and patients’ quality of life [26[•]].

Finally, there is evidence from preclinical studies and BMT patients that endogenous expression is more effective than exogenously administered ADA [1]. Importantly, the altered intracellular and extracellular signaling in patients’ T cell functions was restored in CD4⁺ T-cell lines established from ADA-SCID patients after gene therapy, whereas still present in T cells from patients on PEG-ADA, indicating that the sole extracellular detoxification may not be sufficient to achieve sustained immune recovery [6[•]]. Moreover, the restoration of SAHH activity, sensitive indicator of normalized intracellular metabolite concentrations, and the correction of both proliferative responses and Th1/Th2 cytokine production in T cells generated after gene therapy, demonstrated the ability of ADA gene transfer to correct both the intracellular and extracellular mechanisms of the disease [6[•]].

Potential drawbacks of gene therapy

Integrating vectors carry an inherent risk of insertional mutagenesis associated with retroviral vector insertions in sensitive genomic sites [27]. By inserting into such regions, the strong enhancer elements found in the viral LTR can lead to aberrant gene expression, causing clonal expansion or leukemic proliferation, as occurred in the case of chronic granulomatous disease [28] and SCID-X1 [29,30]. The cumulative experience of our study [11^{••},31] as well as of other clinical trials did not reveal such complications or other adverse events [3,25[•]], indicating that ADA-SCID gene therapy has a favorable risk/benefit profile. Unique risk factors may have contributed to the different outcomes of the chronic granulomatous disease or SCID-X1 trials, such as vector constructs or promoters, inappropriate expression of transgenes involved in cell signaling [25[•]], cooperation between transgene and cellular oncogenes [32], or disease background associated with high rate of transformation [33].

Vector integration profile in patients treated with gene therapy

Two recent studies assessed the retroviral integration profile in our ADA-SCID gene therapy trial. Clonal analysis of long-term repopulating cell progeny 1.5–47 months after autologous transplantation revealed polyclonal T-cell populations, with up to 121 different gene corrected progenitors contributing to T lymphopoiesis [31]. Importantly, the presence of shared vector integrations among multiple hematopoietic lineages demonstrated stable engraftment of multipotent HSC. Among others, retroviral vectors integrations occurred within and/or near LMO2, BLM, CCND2 and BCL2, known potentially oncogenic loci, but did not result in selection or expansion of malignant cell clones *in vivo* [31].

Using quantitative transcript analysis of genes close to integrated proviruses we recently extended these analyses, providing a direct estimate of the transcriptional interference caused by vector integration at single-cell level [34]. Results from 120 genes within a 200kb-window around integration sites showed modest dysregulation of around 6% of genes in 18.6% of the T-cell clones compared with controls. Interestingly, the extent of altered gene expression detected in our cohort of *ex vivo* derived clones was significantly lower as compared to that induced by RIS in T-cells cloned after in-vitro transduction [35], or in leukemic cells from patients of the SCID-X1 and CGD trial [28–30].

Nonetheless, affected clones maintained a stable phenotype and no measurable effects were observed on the biology of the bulk population, suggesting that the utilized gene therapy protocol does not cause major alterations of gene expression in T lymphocytes developed after transplant. Despite these encouraging results, the detected retroviral vector integrations into potential oncogenic sites demands continued monitoring of treated individuals in the future.

Future developments of gene therapy

The use of self-inactivating (SIN) lentiviral vectors might provide a superior integration and safety profile for ADA-SCID gene therapy [36[•]]. Lentiviral vectors have elicited great interest for their ability to transduce nondividing cells and especially for highly efficient transduction of HSCs. In terms of safety, the integration profile of lentiviral vectors, although predominantly intragenic, does not target the promoter regions of active genes to the same extent as retroviral vectors. In a SIN configuration, together with transgene expression from a physiological nonviral internal promoter, the potential for insertional gene activation from SIN-lentiviral vectors is likely to be significantly reduced. In comparative experiments in tumor prone murine strains, SIN-lentiviral vectors resulted in reduced tumorigenesis compared with non-SIN retroviral vectors, similar to those used in existing ADA-SCID gene therapy trials [36[•]].

Vector development for ADA deficiency in preclinical mouse models recently progressed to the use of SIN-lentiviral vectors. Our laboratory showed the efficacy of a SIN-lentiviral vector driving ADA expression from the phosphoglycerate kinase (PGK) promoter [37]. Bone marrow cells of ADA^{-/-} mice were transduced *ex vivo* and injected intravenously (i.v.), resulting in full metabolic detoxification, restoration of ADA activity, differentiation and immune functions of lymphoid cells, including antigen-specific antibody production. In our experience, pretransplant conditioning was crucial for long-term survival of ADA^{-/-} mice, as animals receiving

gene therapy or allogeneic transplant without irradiation died due to poor engraftment.

Contrarily, Carbonaro *et al.* [38[•]] showed that 1–3% of donor engraftment achieved without prior cytoreductive conditioning was sufficient to rescue newborn mice. Relatively low doses of total body irradiation increased engraftment levels significantly underlining that ADA-deficient SCID is unique in its responses to nonmyeloablative conditioning. In a different approach, a SIN-lentiviral vector driving ADA expression through the MND enhancer/promoter was used to treat neonatal ADA^{-/-} mice directly *in vivo* by i.v. injection [39]. In addition to prolonged survival, mice showed significantly increased lymphoid cells counts and reconstitution of mitogen-induced T-cell proliferation. However, due to a lack of HSC transduction by the systemically administered lentiviral vectors, no selective advantage of gene-corrected T cells was observed. In comparison to ex-vivo transduction of HSC this will likely hinder the future clinical application of this approach.

Although lentiviral vectors have been widely predicted to be a safer backbone than retroviral vector, a relevant biological observation has been recently reported in a French gene therapy trial for patients with thalassemia. A relative clonal dominance that appears to be the result of vector integration into a gene encoding the DNA-binding protein HMGA2 was observed in a patient treated with lentiviral-mediated HSC gene therapy [40]. Further investigations are needed to clarify the nature and evolution of this finding and its association with the specific vector design and thalassemic disease background.

Conclusion

In the past decades gene therapy for ADA-SCID has not only evolved significantly but also has reached a stage where it has ‘fulfilled its promise’ [5[•]]. Basic science and clinical research cooperated in order to improve therapeutic options for this otherwise fatal disease. HSC gene therapy for ADA-SCID has been shown to be well tolerated and efficacious over almost two decades of follow-up. Nonetheless, it is likely that lifelong monitoring of the treated individuals will be necessary to exclude oncogenic events due to the detected retroviral vector integrations. The future development of a SIN-lentiviral vector based protocol might provide a superior integration and safety profile for ADA-SCID gene therapy.

As reviewed recently by Gaspar *et al.* [41^{••}] ADA-SCID remains a challenging condition to treat. The availability of different management options without formal outcome studies has made the choice of treatment difficult. BMT from an HLA-identical sibling donor remains the

treatment of choice. With large-scale outcome studies still lacking, the choice between lifelong ERT, unrelated BMT and gene therapy should be based on the risk/benefit ratio, the availability and costs of the three different treatment options. Given the long-term experience in gene therapy [11^{••}], this treatment can now be considered in all patients lacking a matched sibling donor. Further issues remain regarding the best treatment for the autoimmune and nonimmune manifestations of ADA-SCID and particularly the cognitive and neurological defects [41^{••}]. Additional studies will have to dissect the contribution of immunodeficiency from that of the metabolic disease, in order to allow adjustment of current therapeutic options and improve patients' long-term prospects.

Acknowledgement

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Hirschhorn 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. pp. 169–196.
- 2 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.
- 3 Booth C, Hershfield M, Notarangelo L, *et al.* Management options for adenosine deaminase deficiency; proceedings of the EBMT satellite workshop (Hamburg, March 2006). *Clin Immunol* 2007; 123:139–147.
- 4 Hershfield MS. Combined immune deficiencies due to purine enzyme defects. *Immunologic Disorders in Infants and Children*. 5th ed. Philadelphia: Saunders; 2004:480–504.
- 5 Kohn DB, Candotti F. Gene therapy fulfilling its promise. *N Engl J Med* 2009; 360:518–521.
- Editorial summarizing the development and recent success of ADA-SCID gene therapy with thorough evaluation of its risks and benefits.
- 6 Cassani B, Mirolo M, Cattaneo F, *et al.* Altered intracellular and extracellular signaling leads to impaired T-cell functions in ADA-SCID patients. *Blood* 2008; 111:4209–4219.
- Provides a more clear understanding of the physiopathology of ADA-SCID and confirms the efficacy of ADA-gene transfer in restoring normal metabolic pathways and immunological functions on the cellular level.
- 7 Notarangelo LD, Stoppoloni G, Toraldo R, *et al.* Insulin-dependent diabetes mellitus and severe atopic dermatitis in a child with adenosine deaminase deficiency. *Eur J Pediatr* 1992; 151:811–814.
- 8 Ozsahin H, Arredondo-Vega FX, Santisteban I, *et al.* Adenosine deaminase deficiency in adults. *Blood* 1997; 89:2849–2855.
- 9 Mazzolari E, de Martis D, Forino C, *et al.* Single-center analysis of long-term outcome after hematopoietic cell transplantation in children with congenital severe T cell immunodeficiency. *Immunol Res* 2009; 44:4–17.
- Long-term follow-up of SCID patients after HSCT, underlining that low immune reconstitution is associated with a higher incidence of clinical events or autoimmunity.
- 10 Neven B, Leroy S, Decaluwe H, *et al.* Long-term outcome after haematopoietic stem cell transplantation of a single-centre cohort of 90 patients with severe combined immunodeficiency: long-term outcome of HSCT in SCID. *Blood* 2009; 113:4114–4124.
- Large-scale follow-up of SCID patients after HSCT recommends that late-onset complications, such as autoimmune manifestations, require patient's long-term follow-up.
- 11 Aiuti A, Cattaneo F, Galimberti F, *et al.* Long-term safety and efficacy of gene therapy for adenosine deaminase (ADA)-deficient severe combined immunodeficiency. *New Engl J Med* 2009; 360:447–458.
- First demonstration of clinical efficacy and long-term safety of HSC gene therapy for ADA-SCID, showing that gene therapy has favorable safety profile and is effective in restoring normal purine metabolism and immune functions.
- 12 Sitkovsky M, Lukashev D, Deaglio S, *et al.* Adenosine A2A receptor antagonists: blockade of adenosine effects and T regulatory cells. *Br J Pharmacol* 2008; 153 (Suppl 1):S457–S464.
- 13 Aiuti A, Ficara F, Cattaneo F, *et al.* Gene therapy for adenosine deaminase deficiency. *Curr Opin Allergy Clin Immunol* 2003; 3:461–466.
- 14 Bollinger ME, Arredondo-Vega FX, Santisteban I, *et al.* Brief report: hepatic dysfunction as a complication of adenosine deaminase deficiency. *N Engl J Med* 1996; 334:1367–1371.
- 15 Sauer AV, Mrak E, Jofra Hernandez R, *et al.* ADA-deficient SCID is associated with a specific microenvironment and bone phenotype characterized by RANKL/OPG imbalance and osteoblast insufficiency. *Blood* 2009 (in press).
- 16 Rogers MH, Lwin R, Fairbanks L, *et al.* Cognitive and behavioral abnormalities in adenosine deaminase deficient severe combined immunodeficiency. *J Pediatr* 2001; 139:44–50.
- 17 Honig M, Albert MH, Schulz A, *et al.* Patients with adenosine deaminase deficiency surviving after hematopoietic stem cell transplantation are at high risk of CNS complications. *Blood* 2007; 109:3595–3602.
- 18 Titman P, Pink E, Skucek E, *et al.* Cognitive and behavioral abnormalities in children after hematopoietic stem cell transplantation for severe congenital immunodeficiencies. *Blood* 2008; 112:3907–3913.
- Together with reference 16 suggests that cognitive defects occur as intrinsic consequence of ADA-SCID, rather than as a result of transplantation or previous infection.
- 19 Zhou Y, Mohsenin A, Morschl E, *et al.* Enhanced airway inflammation and remodeling in adenosine deaminase-deficient mice lacking the A2B adenosine receptor. *J Immunol* 2009; 182:8037–8046.
- 20 Blaese RM, Culver KW, Miller AD, *et al.* T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science* 1995; 270:475–480.
- 21 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.
- 22 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.
- 23 Kohn DB, Hershfield MS, Carbonaro D, *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; 4:775–780.
- 24 Ficara F, Superchi DB, Hernandez RJ, *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; 10:1096–1108.
- 25 Kohn DB. Gene therapy for childhood immunological diseases. Bone marrow transplant 2008; 41:199–205.
- Reviewing the future prospects of new gene transfer methods for primary immunodeficiencies.
- 26 Aiuti A, Brigida I, Ferrua F, *et al.* Hematopoietic stem cell gene therapy for adenosine deaminase deficient-SCID. *Immunol Res* 2009; 44:150–159.
- Summarizing the development and clinical trials for gene therapy in ADA-deficient SCID since 1991.
- 27 Baum C, Kustikova O, Modlich U, *et al.* Mutagenesis and oncogenesis by chromosomal insertion of gene transfer vectors. *Hum Gene Ther* 2006; 17:253–263.
- 28 Ott MG, Schmidt M, Schwarzwaelder K, *et al.* Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EV11, PRDM16 or SETBP1. *Nat Med* 2006; 12:401–409.
- 29 Hacein-Bey-Abina S, Garrigue A, Wang GP, *et al.* Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 2008; 118:3132–3142.
- 30 Howe SJ, Mansour MR, Schwarzwaelder K, *et al.* Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest* 2008; 118:3143–3150.
- 31 Aiuti A, Cassani B, Andolfi G, *et al.* Multilineage hematopoietic reconstitution without clonal selection in ADA-SCID patients treated with stem cell gene therapy. *J Clin Invest* 2007; 117:2233–2240.
- 32 Dave UP, Akagi K, Tripathi R, *et al.* Murine leukemias with retroviral insertions at Lmo2 are predictive of the leukemias induced in SCID-X1 patients following retroviral gene therapy. *PLoS Genet* 2009; 5:e1000491.

- 33 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 U S A* 2006; 103:11730–11735.
- 34 Cassani B, Montini E, Maruggi G, *et al.* Integration of retroviral vectors induces minor changes in the transcriptional activity of T cells from ADA-SCID patients treated with gene therapy. *Blood* 2009 (in press).
- 35 Recchia A, Bonini C, Magnani Z, *et al.* Retroviral vector integration deregulates gene expression but has no consequence on the biology and function of transplanted T cells. *Proc Natl Acad Sci U S A* 2006; 103:1457–1462.
- 36 Montini E, Cesana D, Schmidt M, *et al.* The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. *J Clin Invest* 2009; 119:964–975.
- Exchanging genetic elements between retroviral vectors and LVs reveals that transcriptionally active long terminal repeats are major determinants of genotoxicity in gene therapy vectors.
- 37 Mortellaro A, Hernandez RJ, Guerrini MM, *et al.* Ex vivo gene therapy with lentiviral vectors rescues adenosine deaminase (ADA)-deficient mice and corrects their immune and metabolic defects. *Blood* 2006; 108:2979–2988.
- 38 Carbonaro DA, Jin X, Cotoi D, *et al.* Neonatal bone marrow transplantation of ADA-deficient SCID mice results in immunological reconstitution despite low levels of engraftment and an absence of selective donor T lymphoid expansion. *Blood* 2008; 111:5745–5754.
- Significant increase of donor engraftment after low-dose TBI suggests that ADA-deficient SCID is unique in its responses to nonmyeloablative conditioning before bone marrow transplant.
- 39 Carbonaro DA, Jin X, Petersen D, *et al.* In vivo transduction by intravenous injection of a lentiviral vector expressing human ADA into neonatal ADA gene knockout mice: a novel form of enzyme replacement therapy for ADA deficiency. *Mol Ther* 2006; 13:1110–1120.
- 40 Williams DA. Gene therapy continues to mature and to face challenges. *Mol Ther* 2009; 17:1305–1306.
- 41 Gaspar HB, Aiuti A, Porta F, *et al.* How I treat ADA deficiency. *Blood* 2009
- (in press).
- Review of the currently available evidence and thorough evaluation of the treatment options available for ADA-SCID, aimed at providing a consensus management strategy for this condition.