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REVIEW

Update on gene therapy for immunodeficiencies

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Abstract Primary immune deficiencies (PID) are due to blood cell defects and can be treated with transplantation of normal hematopoietic stem cells (HSC) from another person (allogeneic). Gene therapy in which a patient's autologous HSC are genetically corrected represents an alternative treatment for patients with PID, which could avoid the immunologic risks of allogeneic HSCT and confer similar benefits. Recent clinical trials using gene therapy have led to immune restoration in patients with X-linked severe combined immune deficiency (XSCID), adenosine deaminase (ADA)-deficient SCID and chronic granulomatous disease (CGD). However, severe complications arose in several of the patients in whom the integrated retroviral vectors led to leukoproliferative disorders. New approaches using safer integrating vectors or direct correction of the defective gene underlying the PID are being developed and may lead to safer and effective gene therapy for PID.
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Introduction

Conceptual Basis for Considering Gene Therapy for Primary Immune Deficiencies (PID)

The primary immune deficiencies (PID) result from inherited mutations in genes required for the production, function or survival of specific leukocytes (e.g., T, B or NK lymphocytes, neutrophils, antigen-presenting cells). Because these leukocytes are produced from the pluripotent hematopoietic stem cells (HSC) in the bone marrow, allogeneic bone marrow transplantation (BMT) (from a healthy donor) into a patient with a PID can lead to immune restoration. BMT was first reported for the treatment of patients with severe combined immune deficiency (SCID) and Wiskott-Aldrich Syndrome (WAS) in 1968 [1,2]. Since that time, hematopoietic stem cell transplantation (HSCT—a term used to encompass several possible clinical sources of HSC, including bone marrow, mobilized peripheral blood and umbilical cord blood) has been performed for many of the life-threatening PID, including SCID, Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD), leukocyte adhesion deficiency (LAD), X-linked hyper IgM syndrome (X-HIM, due to deficiency of the CD40 ligand CD154), hemophagocytic lymphohistiocytosis (HLH) and immunodysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX), among others [3,4]. While SCID patients may be treated by simply infusing some donor bone marrow cells without prior ablation of their bone marrow or their immune system with chemotherapy, all of the other PID require pretransplant conditioning, full or at least partial marrow cytoreduction, to "make space" in the marrow for the transplanted HSC, and potent immune ablation to prevent rejection of the donor HSC. While allogeneic HSCT may lead to essentially normal immune function and excellent quality of life for patients with severe PID, there are significant risks, both from the conditioning regimen, as well as from the immunologic consequences such as graft-vs.-host disease (GVHD) wherein donor lymphocytes transplanted with the HSC source react against the recipient's tissues. Advances in transplantation immunology and graft engineering (e.g., depletion of specific subpopulations of T cells capable of causing graft-vs.-host disease or addition of regulatory T cells) are likely to improve the efficacy and safety of allogeneic HSCT from fully and partially matched donors for patients with PID.

The concept underlying gene therapy for PID is that gene correction and re-transplantation of the patient's own HSC could lead to the same clinical benefits as allogeneic HSCT, but without the immunologic complications. Efforts to perform gene therapy for PID began in the 1980s as gene delivery vectors were derived from murine retroviruses. Retroviral vectors can be produced through recombinant DNA techniques to carry the cDNA for a human gene with high efficiency into target cells, such as HSC or T lymphocytes, and integrate the gene into the chromosomal DNA of the target cell (transduction), where it can be faithfully passed on to successive generations of daughter cells during mitosis. Stable persistence of the corrective gene is essential when targeting cells such as HSC that will undergo massive proliferation to produce the mature progeny cells. Retroviral vectors integrate relatively randomly into the genome of target cells, so that the vector is present in a characteristic site in the clonal descendants of each HSC that is transduced. Studies performed at that time demonstrated the ability of retroviral vectors to introduce genes into the pluripotent HSC from murine bone marrow, based on the presence of vector sequences present in the same integration site in both myeloid and lymphoid cells of the recipient [5,6]. Based on these results, an initial wave of enthusiasm emerged for the prospects of gene therapy to be used to treat a host of diseases, spreading from the inherited blood cell diseases to other genetic diseases and acquired conditions including cancer, cardiovascular disorders, neurological diseases, and others.

Adenosine Deaminase (ADA)-Deficient SCID—Initial Trials

Following an initial study in which retroviral vectors were used to insert a marker gene into tumor-infiltrating T lymphocytes to allow them to be tracked *in vivo*, the first clinical trial for gene therapy was developed at the National Institutes of Health (NIH), targeting peripheral blood T lymphocytes from patients with adenosine deaminase (ADA)-deficient SCID [7,8]. ADA-deficiency was the first genetic cause of human SCID to be characterized at the biochemical and genetic basis, and normal human ADA cDNA were cloned in the mid-1980s [9,10]. Retroviral vector technology had advanced such that vectors could be made to moderately high titers at a scale sufficient to be used to treat the numbers of cells that would be obtained from patients without the production of contaminating replication-competent virus that could spread within a patient and possibly cause disease [11]. The NIH group used a retroviral vector carrying a normal human ADA cDNA to transduce T cells from the peripheral blood of patients with ADA-deficient SCID who were receiving ADA enzyme replacement therapy, which partially restored the numbers of circulating T lymphocytes. The protocol called for repeated cycles of leukopheresis, ADA gene transduction and cell reinfusion.

Two children with ADA-deficient SCID were treated under this protocol beginning in 1990. There were no complications from the procedures and they received multiple cycles of cell treatment. The presence of circulating T cells expressing ADA enzyme activity was demonstrated and this has persisted in one of them for at least 12 years of follow-up [12]. However, it is not apparent whether any clinical benefit was derived from the procedure, as the patients remained on the ADA enzyme replacement therapy. Two other trials targeting peripheral blood T cells from ADA-deficient SCID patients were performed, but again no conclusive benefits could be attributed [13–15].

Subsequent studies have primarily focused on targeting gene correction into HSC, which may lead to long-term production of a broad repertoire of functioning lymphocytes. The first clinical trial to introduce a gene into human HSC also involved a marker gene, in this case for pediatric patients with leukemia or neuroblastoma undergoing autologous HSCT [16]. The goal was to determine if residual malignant cells present in the transplant inoculums contributed to posttransplant relapses, which would be evidenced by the presence of the marker gene in some cells at relapse.

In fact, marked cells at relapse were seen in several patients, indicating the need to purge the grafts of contaminating tumor cells prior to reinfusion. Normal circulating white blood cells containing the marker gene were present at very low frequencies.

Bordignon and colleagues at the San Raffaele Hospital in Milan then applied the methods of retroviral-mediated gene transfer to hematopoietic stem cells, isolating them from the patients' bone marrow, culturing them for a few days for gene addition, and then reinfusing the cells into the patients [13]. Again, while the procedure did not have complications, there was no evidence of clinical benefit and only very low levels of gene-containing peripheral blood leukocytes were produced. Two other trials targeting the HSC in bone marrow or umbilical cord blood of infants diagnosed in utero with ADA-deficient SCID were performed with no evidence of efficacy [17,18].

In retrospect, the lack of success from these early trials is not surprising. The methods used for culturing the HSC for retroviral transduction are now known to be suboptimal both for gene transfer and for survival of the HSC. Additionally, the continued administration of ADA enzyme replacement therapy may have blunted the putative selective advantage conferred to ADA-corrected lymphocytes, impeding the potential amplification of initial gene correction efficiency.

During the 1990s there was ongoing research on improving the methods for gene transfer to human HSC, and retroviral vectors were produced at higher titers capable of more efficient gene delivery [19]. New hematopoietic growth factors were identified that were able to induce cycling of primitive long-lived HSC, the ideal targets for gene therapy, which is required for retroviral-mediated gene transfer [20]. The presence of either a feeder layer of marrow stromal cells or the extracellular matrix protein fibronectin was shown to both enhance gene transfer to human HSC as well as to support their survival during *ex vivo* culture [21]. These improved techniques were applied for a second generation of clinical trials of gene therapy for PID begun in the late 1990s.

Second-Generation Trials of Gene Therapy for ADA-Deficient SCID

The group at the San Raffaele Telethon Institute for Gene Therapy, Milan began a trial of retroviral-mediated gene transfer for ADA-deficient SCID in 2000, using the improved transduction methods. They also made two major changes from the prior clinical approach. They treated two ADA-deficient SCID patients who were not receiving ADA enzyme replacement therapy, due to financial constraints in their countries of origin [22]. Absence of enzyme therapy would allow the selective advantage for the gene-corrected cells to be manifest. They had previously observed that a child treated with ADA gene transfer to peripheral blood T lymphocytes had higher level of gene-containing T lymphocytes when off ADA enzyme replacement than when on it, suggesting that enzyme replacement blunted the survival advantage of ADA gene-corrected cells. Additionally, they were treated with pretransplant conditioning by administration of the chemotherapy agent busulfan, in approximately one fourth of the full dosage typically used in clinical BMT to achieve complete marrow ablation. Busulfan is an alkylating agent that is highly specific for eradicating HSC, and acts to "make space" in the marrow to facilitate engraftment of transplanted HSC.

Both subjects has recovery of their immune function; one has had sustained protective immunity, whereas the second who had a smaller HSC dosage only achieved partial immune reconstitution. These results represent a major milestone in the treatment of PID. This group recently reported extended outcome results in a total of 10 subjects treated under this protocol, with the majority of the patients achieving clinically beneficial immune reconstitution that has allowed them to lead essentially normal lives without the need for ADA enzyme replacement therapy [23]. Similar studies at University College London, Institute of Child Health in the United Kingdom and by our group in the U.S. at the University of California, Los Angeles and the National Institutes of Health are obtaining similar results [24,25]. We have observed immune reconstitution in the majority of the treated subjects who received busulfan and were not on ADA enzyme replacement (T lymphocytes rising to 300–500/mm^3 within 1 year).

Gene Therapy for X-Linked SCID (XSCID)

The X-linked form of SCID (XSCID) was determined to result from inherited defects in the gene encoding a component of the receptor for multiple cytokines acting to promote lymphocyte development and function, called the common cytokine receptor gamma chain or γc [26]. A strong selective advantage for lymphoid progenitor cells in XSCID patients was demonstrated by a patient who underwent a spontaneous reversion of a γC mutation in a single lymphoid progenitor cell, leading to some immunologic reconstitution. Preclinical studies showed that retroviral-mediated transfer of a normal human γc cDNA into cells from XSCID patients or murine models of the disease restored lymphocyte production and activity [27].

Based on these results, a clinical trial of gene therapy for XSCID was initiated at the Hôpital Necker Enfant-Malade in Paris, France. Again, HSC from the patients' bone marrow was cultured for retroviral-mediated gene transfer and then reinfused into the patients. In this trial, no chemotherapy conditioning was administered, with the expectation that the gene-corrected lymphoid cells would have a very high selective survival in the profoundly lymphopenic patients, and marrow cytoreduction to facilitate higher engraftment of HSC would not be needed.

The results in the first two subjects were reported in 2000, showing rapid and robust production of T lymphocytes, with lesser improvements in the numbers of circulating B and NK cells [28]. Subsequent reports demonstrated similar responses in 9 of 10 subjects, with the exception being a patient who had marked organomegaly at the time of treatment, which may have led to consumption of the infused cells [29]. A trial at UCL Institute of Child Health using similar techniques achieved immune reconstitution in another 10 subjects [30].

However, 2–5 years after the treatment, a serious complication developed in a total of 5 of the 20 subjects treated in the two trials, with a leukemia-like T lymphoproliferative

disorder arising [31,32]. Patients who had been clinically stable with good immune function developed relatively abrupt onset of escalating levels of circulating T lymphocytes, with thymic mass and organomegaly. They were treated with chemotherapy and four have remained in complete remission with continued restored immunity, but one succumbed from the leukemia, despite therapy.

Investigations into the mechanisms found that in each subject, there was an outgrowth of a clonal population of T cells containing the retroviral vector integrated adjacent to one or more cellular proto-oncogenes (LMO-2 in 4 of the cases) [33,34]. Retroviruses are known to integrate into the chromosomal DNA of target cells in a relatively random manner, such that each HSC that was transduced would have the vector at a different location. Retroviruses are also known to be capable of *trans*-activating the expression of cellular genes in the vicinity of their integration by the action of the viral enhancers contained in the viral long-terminal repeats (LTR); if the cellular genes activated are ones that control cellular growth (proto-oncogenes), the result can be stimulated proliferation that can lead to accumulation of additional growth-promoting mutations and eventually malignant transformation. This, in fact, is the mechanism by which the oncogenic leukemia-causing retroviruses cause disease in susceptible animal hosts when capable of unfettered replication in infected neonates resulting in large numbers of integration events. Presumably, the initial gene transfer to the patients' HSC led to thousands or even millions of vector integrants at sites across the genome in individual cells; rare integrants that *trans*-activated cellular proto-oncogenes induced increased cell proliferation which then led to a cascade of cellular events culminating in the malignant transformation. The reasons why this complication has occurred in 5 of 20 XSCID patients, but none of more than 20 ADA-deficient SCID patients are not clear. The role of the γc gene product (a *trans*-membrane protein capable of providing intracellular signaling), the nature of the XSCID patients' marrow stem cells, effects of the γc-deficiency on the susceptibility to transformation and the rapidity of immune reconstitution in the XSCID subjects have all been speculated as involved, but no definitive explanation has yet emerged.

One other trial for XSCID has been reported that treated three teenage boys with XSCID who had prior unsuccessful allogeneic HSCT and had poor immunity and other chronic complications [35]. Only one of these subjects had any evidence of improvement in immune function, suggesting that this gene therapy approach may be most effective in younger subjects with potential for robust thymic function. One patient with SCID due to deficiency of the Janus kinase 3 was treated in a clinical trial led by Brian Sorrentino at St. Jude Children's Research Hospital without clinical benefit.

Gene Therapy for Chronic Granulomatous Disease (CGD)

Parallel efforts were made to perform gene therapy for chronic granulomatous disease (CGD) in trials performed at the National Institutes of Health and Indiana University School of Medicine in the mid-late 1990s. They used *ex vivo* gene transfer with γ-retroviral vectors to peripheral blood stem cells (PBSC) mobilized by granulocyte-colony stimulating factor (G-CSF), which were reinfused without prior treatment with chemotherapeutic agents [36–38]. Low frequencies of corrected granulocytes were seen in the peripheral blood for the first months in a few of the subjects, but no long-lasting effects were seen.

Subsequently, a trial was performed in Germany in which a moderate dosage of busulfan (8 mg/kg) was given prior to reinfusion of the transduced PBSC [39]. The two young men treated had severe infections that had persisted despite intensive medical therapy. These infections resolved after the gene therapy procedure, as the patients made neutrophils with functional oxidase activity. Relatively high levels of corrected leukocytes were seen in peripheral blood (~20%) in the first months after the gene therapy procedure and these rose to as high as 80% over the first year. The vector integration sites were studied in the CGD patients and revealed a highly restricted pattern, with the majority of vector integrants in the engrafted stem cells being near one of a few genes known to be involved in myeloid cell proliferation (MDS-1, PRDM16 or SETBP1). These two patients went on to develop myelodysplasia, a preleukemic condition; one subject has had a bone marrow transplant and the other died of an acute infection with a loss of the restored neutrophil function. The underlying mechanisms for the oligoclonal expansion and progression to myelodysplasia are not fully elucidated, but the retroviral vector used had an LTR with potent enhancer activity in myeloid progenitor cells and this may have led to *trans*-activation of genes that promote myeloid cell proliferation. The use of PBSC target cells that were stimulated by the myeloid growth factor G-CSF may have presented a large number of transcriptionally active myeloid-proliferative genes as integration targets. As with the XSCID trials, it will be vital to understand how to avoid this unwanted complication while retaining the clear-cut clinical benefits that can be attained.

Gene Therapy for Leukocyte Adhesion Deficiency (LAD)

A clinical trial was performed for leukocyte adhesion deficiency (LAD), due to deficiency of the CD18 adhesion protein. Two patients were treated using a retroviral vector but without chemotherapy conditioning [40]. Essentially, no gene-transduced lymphocytes were produced in the patients. More recently, these investigators have reported promising results in a canine model of LAD, using a foamy virus vector and low dose total-body irradiation (200 cGy) as conditioning, with excellent restoration of lymphocyte function [41].

Gene Therapy for Wiskott-Aldrich Syndrome (WAS)

Wiskott-Aldrich syndrome (WAS) is another PID that can be treated with allogeneic HSCT [42]. Results using matched sibling donors, especially for young subjects, have been excellent. Outcomes using parental (haploidentical) donors were less favorable, but more recent transplants using matched unrelated donors have led to improved results, again best in boys treated under 5 years of age in generally good clinical health [43]. Nevertheless, gene therapy has the

potential to provide even better results, by avoiding the immunologic risks of rejection and graft-vs.-host disease. Trials using retroviral and lentiviral vectors carrying a normal human WASP cDNA are beginning in Europe, with others under development in the U.S.

One important issue unique to WAS relates to the high incidence of autoimmune complications in WAS patients undergoing allogeneic HSCT, which occur with greatest frequency in those who have split chimerism and a large component of residual host leukocytes after transplant [44]. If uncorrected WAS lymphocytes can act in a dysregulated manner, even in the presence of some normal lymphocytes, it may be necessary to achieve full ablation of their marrow prior to gene therapy to avoid autoimmune complications.

Development of Safer Vectors for Gene Therapy of PID

The serious complications which arose in the XSCID and CGD trials have highlighted the risks from the types of integrating γ-retroviral vectors used, which contain potent enhancers in their LTR that can *trans*-activate cellular genes. Besides the efforts that have ensued to understand these risks, new vectors have been developed to improve the safety profile. Early attempts to make retroviral vectors in which the enhancers of the LTR "self-inactivate" (so-called "SIN" vectors) were hampered by resultant low titers and poor gene expression. Primarily through the efforts of Christopher Baum and coworkers in Hannover, Germany, to carefully dissect the elements of the retroviral vector backbone, new SIN retroviral vectors were developed that can be made at titers high enough for clinical applications [45]. This group has also shown that using some specific cellular promoters to drive expression of the transgenes (e.g., from the elongation factor-1 alpha and the phosphoglycerate kinase gene) can lead to expression of most cDNA at levels sufficient to correct the cellular defects with significantly decreased risks of *trans*-activation of adjacent cellular genes [46]. These investigators with colleagues in France, the UK and the U.S. are developing clinical trials for XSCID that would use this type of improved γ-retroviral vector, with the expectation that it will be as effective as the earlier vector with greatly decreased risk for insertional transformation.

Another major advance in gene delivery vectors has been the development of gene transfer vectors based on the Lentiviral class of retroviruses. Lentiviruses, including the HIV-1 virus, are capable of more effective gene transfer to human HSC than γ-retroviral vectors and will perform this function with a much shorter time of cell culture (1 day vs. 4), which may help preserve the engraftment capacity of the stem cells [47,48]. Importantly, it has been possible to produce SIN versions of lentiviral vectors lacking LTR enhancers and carrying cellular promoters. Studies using *in vitro* and *in vivo* models to measure risks of insertional transformation show that SIN lentiviral vectors with cellular promoters have markedly decreased risks compared to the first generation of γ-retroviral vectors with their intact LTR [49,50]. "Third-generation" clinical trials of gene therapy for the PID are beginning using these putatively safer retro- and lentiviral vectors.

Gene Correction Using Homologous Recombination (HR)

All of the studies described above have used gene addition approaches, with viral-mediated insertion of the relevant cDNA. While benefits have been obtained, complications from the randomly integrated vectors have arisen as described. Although the newer retro- and lentiviral vectors may improve the therapeutic index, risks of insertional *trans*-activation may persist. Additionally, some gene products may require strictly regulated expression for safety and efficacy. For example, Brenner and colleagues observed that constitutive expression of CD154 in a murine model of X-linked Hyper IgM led to lymphomatous transformation [51]. Presumably, safe expression of CD154 will need to follow the endogenous pattern of transient expression upon T-cell activation, followed by down-regulation to avoid overstimulation. It has proven difficult to produce vectors carrying complex transcriptional control elements to provide physiologic expression of transgenes transferred by integrating vectors.

Therefore, efforts are underway to develop robust methods for direct *gene correction* of the endogenous defective sequences. Direct repair of the disease-causing mutations would allow expression of the normal gene product under control of all of the physiologic mechanisms that govern transcription in various states of cell differentiation and activation. Gene correction may be achieved by harnessing the cellular pathways for homologous recombination (HR), one of the major mechanisms for repairing DNA damage, such as double-stranded breaks, which occur normally during chromosome replication and as a result of exposure to radiation, ultraviolet light and some chemicals [52]. The HR pathway uses a second copy of the broken gene, usually contained on the sister chromosome, to serve as a template to repair a patch of DNA surrounding the DNA break. Adding to cells a segment of DNA or RNA carrying the normal sequence complementary to the region of a gene containing the disease-causing mutation, can lead to gene repair, as the added "donor" sequence is used as a template for HR-mediated repair. However, such HR driven by addition of donor sequences occurs at a very low frequency (<1/10^6 cells), which would not be clinically useful.

Current work focuses on the use of specific proteins to make targeted double strand breaks in the DNA near pathogenic mutations to augment the frequency of HR driven by added donor sequences. Zinc finger nucleases (ZFN) are synthetic fusion proteins that combine multiple zinc finger motifs, each capable of binding to specific DNA sequences, with a nuclease that can introduce a DNA break [53]. Naturally occurring homing endonucleases are a class of proteins, mostly present in yeast, that bind to specific DNA sequences and introduce DNA breaks; studies are seeking to understand how these homing endonucleases choose their DNA targets to attempt to impose new specificities so that they will act on disease-related loci [54,55]. Experimental results in cell lines and primary cells have shown that the use of these approaches to introduce a targeted DNA break can increase the frequency of HR-mediated gene repair to as high as 1–10% of cells, which is approaching the range that would be clinically beneficial for PID [56]. As envisioned, HR would

be performed *in vitro* by adding the gene-correcting donor sequences and the targeted endonuclease to HSC, where they would act in a "hit and run" manner to correct permanently the patient's mutation, but these reagents would not persist in the cells. The efficacy and potential genotoxicity of these gene correction methods are being studied and, if safe and effective, could supplant gene addition approaches for gene therapy of PID and other inherited disorders.

The Future of Gene Therapy for Other PID

Gene therapy represents an alternative to allogeneic HSCT for patients with PID, which is progressing to more effective approaches. Despite the initial successes that have been achieved, there have been some serious complications. Additional challenges remain to the more broad application of gene therapy for PID. The efficiency with which hematopoietic stem cells are gene corrected, either by gene addition or gene correction, remain low and limiting for most disorders, other than those for which profound selective amplification occurs, as in XSCID. Improved vectors, such as those derived from lentiviruses, or gene correction approaches are being advanced to the clinic. If these newer methods do lead to continued efficacy and greater safety of gene therapy, the spectrum of PID that may be approached by gene therapy will broaden. Other genetic forms of SCID (e.g., Jak3 deficiency, Rag, Artermis, etc), CGD, as well as WAS, X-HIM, HLH, XLA, IPEX, etc. could all be treated when methods are in hand for their safe and effective correction. Each specific disorder requires a dedicated effort to produce the relevant reagents, perform the preclinical efficacy and safety studies and develop the clinical trial protocol and reagents. These are relatively expensive activities that take several years to bring to fruition. However, the human as well as medical costs incurred by the severe PID makes these efforts worthwhile and of high importance.

References

[1] R.A. Gatti, H.J. Meuwissen, H.D. Allen, R. Hong, R.A. Good, Immunological reconstitution of sex-linked lymphopenic immunological deficiency, Lancet 2 (1968) 1366–1369.

[2] F.H. Bach, R.J. Albertini, P. Joo, J.L. Anderson, M.M. Bortin, Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome, Lancet 2 (1968) 1364–1366.

[3] C. Antoine, S. Müller, A. Cant, M. Cavazzana-Calvo, P. Veys, J. Vossen, A. Fasth, C. Heilmann, N. Wulffraat, R. Seger, S. Blanche, W. Friedrich, M. Abinun, G. Davies, R. Bredius, A. Schulz, P. Landais, A. Fischer, European Group for Blood and Marrow Transplantation, European Society for Immunodeficiency, long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99, Lancet 361 (2003) 553–560.

[4] L.M. Griffith, M.J. Cowan, D.B. Kohn, L.D. Notarangelo, J.M. Puck, K.R. Schultz, R.H. Buckley, M. Eapen, N.R. Kamani, R.J. O'Reilly, R. Parkman, C.M. Roifman, K.E. Sullivan, A.H. Filipovich, T.A. Fleisher, W.T. Shearer, Allogeneic hematopoietic cell transplantation for primary immune deficiency diseases: current status and critical needs, J. Allergy Clin. Immunol. 122 (2008) 1087–1096.

[5] D.A. Williams, I.R. Lemischka, D.G. Nathan, R.C. Mulligan, Introduction of new genetic material into pluripotent haematopoietic stem cells of the mouse, Nature 310 (1984) 476–480.

[6] J.E. Dick, M.C. Magli, D. Huszar, R.A. Phillips, A. Bernstein, Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/Wv mice, Cell 42 (1985) 71–79.

[7] S.A. Rosenberg, W.F. Anderson, R.M. Blaese, P. Hwu, J.R. Yannelli, J.C. Yang, S.L. Topalian, D.J. Schwartzentruber, J.S. Weber, S.E. Ettinghausen, et al., The development of gene therapy for the treatment of cancer, Ann. Surg. 218 (1993) 455–463.

[8] R.M. Blaese, K.W. Culver, A.D. Miller, C.S. Carter, T. Fleisher, M. Clerici, G. Shearer, L. Chang, Y. Chiang, P. Tolstoshev, J.J. Greenblatt, S.A. Rosenberg, H. Klein, M. Berger, C.A. Mullen, W.J. Ramsey, L. Muul, R.A. Morgan, W.F. Anderson, T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years, Science 270 (1995) 475–480.

[9] E.R. Giblett, J.E. Anderson, F. Cohen, B. Pollara, H.J. Meuwissen, Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity, Lancet 2 (1972) 1067–1069.

[10] D.A. Wiginton, G.S. Adrian, R.L. Friedman, D.P. Suttle, J.J. Hutton, Cloning of cDNA sequences of human adenosine deaminase, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 7481–7485.

[11] A.D. Miller, C. Buttimore, Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production, Mol. Cell. Biol. 6 (1986) 2895–2902.

[12] L.M. Muul, L.M. Tuschong, S.L. Soenen, G.J. Jagadeesh, W.J. Ramsey, Z. Long, C.S. Carter, E.K. Garabedian, M. Alleyne, M. Brown, W. Bernstein, S.H. Schurman, T.A. Fleisher, S.F. Leitman, C.E. Dunbar, R.M. Blaese, F. Candotti, 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 101 (2003) 2563–2569.

[13] C. Bordignon, L.D. Notarangelo, N. Nobili, G. Ferrari, G. Casorati, P. Panina, E. Mazzolari, D. Maggioni, C. Rossi, P. Servida, A.G. Ugazio, F. Mavilio, Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients, Science 270 (1995) 470–475.

[14] A. Aiuti, S. Vai, A. Mortellaro, G. Casorati, F. Ficara, G. Andolfi, G. Ferrari, A. Tabucchi, F. Carlucci, H.D. Ochs, L.D. Notarangelo, M.G. Roncarolo, C. Bordignon, Immune reconstitution in ADA-SCID after PBL gene therapy and discontinuation of enzyme replacement, Nat. Med. 8 (2002) 423–425.

[15] M. Onodera, T. Ariga, N. Kawamura, I. Kobayashi, M. Ohtsu, M. Yamada, A. Tame, H. Furuta, M. Okano, S. Matsumoto, H. Kotani, G.J. McGarrity, R.M. Blaese, Y. Sakiyama, Successful peripheral T-lymphocyte-directed gene transfer for a patient with severe combined immune deficiency caused by adenosine deaminase deficiency, Blood 91 (1998) 30–36.

[16] M.K. Brenner, D.R. Rill, R.C. Moen, R.A. Krance, J. Mirro Jr., W.F. Anderson, J.N. Ihle, Gene-marking to trace origin of relapse after autologous bone-marrow transplantation, Lancet 341 (1993) 85–86.

[17] P.M. Hoogerbrugge, V.W. van Beusechem, A. Fischer, M. Debree, F. le Deist, J.L. Perignon, G. Morgan, B. Gaspar, L.D. Fairbanks, C.H. Skeoch, A. Moseley, M. Harvey, R.J. Levinsky, D. Valerio, Bone marrow gene transfer in three patients with adenosine deaminase deficiency, Gene Ther. 3 (1996) 179–183.

[18] D.B. Kohn, K.I. Weinberg, J.A. Nolta, L.N. Heiss, C. Lenarsky, G.M. Crooks, M.E. Hanley, G. Annett, J.S. Brooks, A. El-Khoureiy, K. Lawrence, S. Wells, K. Shaw, R.C. Moen, J. Bastian, D.E. Williams-Herman, M. Elder, D. Wara, T. Bowen, M.S. Hershfield, C.A. Mullen, R.M. Blaese, R. Parkman, Engraftment of gene-modified cells from umbilical cord blood in neonates with adenosine deaminase deficiency, Nat. Med. 1 (1995) 1017–1026.

[19] A.D. Miller, J.V. Garcia, N. von Suhr, C.M. Lynch, C. Wilson, M.V. Eiden, Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus, J. Virol. 65 (1991) 2220–2224.
[20] M.A. Dao, A.J. Shah, G.M. Crooks, J.A. Nolta, Engraftment and retroviral marking of $CD34^+$ and $CD34^+$ CD38-human hematopoietic progenitors assessed in immune-deficient mice, Blood 91 (1998) 1243–1255.
[21] H. Hanenberg, X.L. Xiao, D. Dilloo, K. Hashino, I. Kato, D.A. Williams, Colocalization of retrovirus and target cells on specific fibronectin fragments increases genetic transduction of mammalian cells, Nat. Med. 2 (1996) 876–882.
[22] A. Aiuti, S. Slavin, M. Aker, F. Ficara, S. Deola, A. Mortellaro, S. Morecki, G. Andolfi, A. Tabucchi, F. Carlucci, E. Marinello, F. Cattaneo, S. Vai, P. Servida, R. Miniero, M.G. Roncarolo, C. Bordignon, Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning, Science 296 (2002) 2410–2413.
[23] A. Aiuti, F. Cattaneo, S. Galimberti, U. Benninghoff, B. Cassani, L. Callegaro, S. Scaramuzza, G. Andolfi, M. Mirolo, I. Brigida, A. Tabucchi, F. Carlucci, M. Eibl, M. Aker, S. Slavin, H. Al-Mousa, A. Al Ghonaium, A. Ferster, A. Duppenthaler, L. Notarangelo, U. Wintergerst, R.H. Buckley, M. Bregni, S. Marktel, M.G. Valsecchi, P. Rossi, F. Ciceri, R. Miniero, C. Bordignon, M.G. Roncarolo, Gene therapy for immunodeficiency due to adenosine deaminase deficiency, N. Engl. J. Med. 360 (2009) 447–458.
[24] H.B. Gaspar, E. Bjorkegren, K. Parsley, K.C. Gilmour, D. King, J. Sinclair, F. Zhang, A. Giannakopoulos, S. Adams, L.D. Fairbanks, J. Gaspar, L. Henderson, J.H. Xu-Bayford, E.G. Davies, P.A. Veys, C. Kinnon, A.J. Thrasher, Successful reconstitution of immunity in ADA-SCID by stem cell gene therapy following cessation of PEG-ADA and use of mild preconditioning, Mol. Ther. 14 (2006) 505–513.
[25] K.L. Shaw, R. Sokolic, C. Choi, et al., Immune reconstitution after gene therapy for adenosine deaminase deficient severe combined immune deficiency (ADA-SCID), 12th Annual Meeting of the American Society of Gene Therapy. San Diego, California, May 27- 30, 2009.
[26] M. Noguchi, H. Yi, H.M. Rosenblatt, A.H. Filipovich, S. Adelstein, W.S. Modi, O.W. McBride, W.J. Leonard, Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans, Cell 73 (1993) 147–157.
[27] H. Hacein-Bey, M. Cavazzana-Calvo, F. Le Deist, A. Dautry-Varsat, C. Hivroz, I. Rivière, O. Danos, J.M. Heard, K. Sugamura, A. Fischer, G. De Saint Basile, Gamma-c gene transfer into SCID×1 patients' B-cell lines restores normal high-affinity interleukin-2 receptor expression and function, Blood 87 (1996) 3108–3116.
[28] M. Cavazzana-Calvo, S. Hacein-Bey, G. de Saint Basile, F. Gross, E. Yvon, P. Nusbaum, F. Selz, C. Hue, S. Certain, J.L. Casanova, P. Bousso, F. Le Deist, A. Fischer, Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease, Science 288 (2000) 669–672.
[29] S. Hacein-Bey-Abina, F. Le Deist, F. Carlier, C. Bouneaud, C. Hue, J.P. De Villartay, A.J. Thrasher, N. Wulffraat, R. Sorensen, S. Dupuis-Girod, A. Fischer, E.G. Davies, W. Kuis, L. Leiva, M. Cavazzana-Calvo, Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy, N. Engl. J. Med. 346 (2002) 1185–1193.
[30] H.B. Gaspar, K.L. Parsley, S. Howe, D. King, K.C. Gilmour, J. Sinclair, G. Brouns, M. Schmidt, C. von Kalle, T. Barington, M.A. Jakobsen, H.O. Christensen, A. Al Ghonaium, H.N. White, J.L. Smith, R.J. Levinsky, R.R. Ali, C. Kinnon, A.J. Thrasher, Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector, Lancet 364 (2004) 2181–2187.
[31] S. Hacein-Bey-Abina, C. von Kalle, M. Schmidt, et al., A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency, N. Engl. J. Med. 348 (2003) 226–255.
[32] S. Hacein-Bey-Abina, C. von Kalle, M. Schmidt, M.P. McCormack, N. Wulffraat, P. Leboulch, A. Lim, C.S. Osborne, R. Pawliuk, E. Morillon, R. Sorensen, A. Forster, P. Fraser, J.L. Cohen, G. de Saint Basile, I. Alexander, U. Wintergerst, T. Frebourg, A. Aurias, D. Stoppa-Lyonnet, S. Romana, I. Radford-Weiss, F. Gross, F. Valensi, E. Delabesse, E. Macintyre, F. Sigaux, J. Soulier, L.E. Leiva, M. Wissler, C. Prinz, T.H. Rabbitts, F. Le Deist, A. Fischer, M. Cavazzana-Calvo, M, LMO2-associated clonal T-cell proliferation in two patients after gene therapy for SCID-X1, Science 302 (2003) 415–419.
[33] S. Hacein-Bey-Abina, A. Garrigue, G.P. Wang, J. Soulier, A. Lim, E. Morillon, E. Clappier, L. Caccavelli, E. Delabesse, K. Beldjord, V. Asnafi, E. MacIntyre, L. Dal Cortivo, I. Radford, N. Brousse, F. Sigaux, D. Moshous, J. Hauer, A. Borkhardt, B.H. Belohradsky, U. Wintergerst, M.C. Velez, L. Leiva, R. Sorensen, N. Wulffraat, S. Blanche, F.D. Bushman, A. Fischer, M. Cavazzana-Calvo, Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1, J. Clin. Invest. 118 (2008) 3132–3142.
[34] S.J. Howe, M.R. Mansour, K. Schwarzwaelder, C. Bartholomae, M. Hubank, H. Kempski, M.H. Brugman, K. Pike-Overzet, S.J. Chatters, K.C. D de Ridder, S. Gilmour, S.I. Adams, K.L. Thornhill, F.J. Parsley, R.E. Staal, D.C. Gale, J. Linch, L. Bayford, M. Brown, C. Quaye, P. Kinnon, D.K. Ancliff, M. Webb, C. Schmidt, H.B. von Kalle, A.J. Gaspar, Thrasher, Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients, J. Clin. Invest. 118 (2008) 3143–3150.
[35] J. Chinen, J. Davis, S.S. De Ravin, B.N. Hay, A.P. Hsu, G.F. Linton, N. Naumann, E.Y. Nomicos, C. Silvin, J. Ulrick, N.L. Whiting-Theobald, H.L. Malech, J.M. Puck, Gene therapy improves immune function in preadolescents with X-linked severe combined immunodeficiency, Blood 110 (2007) 67–73.
[36] H.L. Malech, P.B. Maples, N. Whiting-Theobald, G.F. Linton, S. Sekhsaria, S.J. Vowells, F. Li, J.A. Miller, E. DeCarlo, S.M. Holland, S.F. Leitman, C.S. Carter, R.E. Butz, E.J. Read, T.A. Fleisher, R.D. Schneiderman, D.E. Van Epps, S.K. Spratt, C.A. Maack, J.A. Rokovich, L.K. Cohen, J.I. Gallin, Prolonged production of NADPH oxidase-corrected granulocytes after gene therapy of chronic granulomatous disease, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 12133–12138.
[37] H. Malech, M. Horwitz, G. Linton, N. Theobald-Whiting, M. Brown, C. Farrell, R. Butz, C. Carter, E. DeCarlo, J. Miller, D. Van Epps, E. Read, T. Fleisher, Extended production of oxidase normal neutrophils in X-linked chronic granulomatous disease (CGD) following gene therapy with gp91phox transduced $CD34^+$ cells, Blood 92 (1998) 690.
[38] C.N. Barese, W.S. Goebel, M.C. Dinauer, Gene therapy for chronic granulomatous disease, Expert Opin. Biol. Ther. 4 (2004) 1423–1434.
[39] M.G. Ott, M. Schmidt, K. Schwarzwaelder, S. Stein, U. Siler, U. Koehl, H. Glimm, K. Kühlcke, A. Schilz, H. Kunkel, S. Naundorf, A. Brinkmann, A. Deichmann, M. Fischer, C. Ball, I. Pilz, C. Dunbar, Y. Du, N.A. Jenkins, N.G. Copeland, U. Lüthi, M. Hassan, A.J. Thrasher, D. Hoelzer, C. von Kalle, R. Seger, M. Grez, Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1, Nat. Med. 12 (2006) 401–409.
[40] T.R. Bauer Jr., D.D. Hickstein, Gene therapy for leukocyte adhesion deficiency, Curr. Opin. Mol. Ther. 2 (2000) 383–388.
[41] T.R. Bauer Jr., J.M. Allen, M. Hai, L.M. Tuschong, I.F. Khan, E.M. Olson, R.L. Adler, T.H. Burkholder, Y.C. Gu, D.W. Russell, D.D. Hickstein, Successful treatment of canine leukocyte adhesion deficiency by foamy virus vectors, Nat. Med. 14 (2008) 93–97.

[42] R. Parkman, J. Rappeport, R. Geha, J. Belli, R. Cassady, R. Levey, D.G. Nathan, F.S. Rosen, Complete correction of the Wiskott-Aldrich syndrome by allogeneic bone-marrow transplantation, N. Engl. J. Med. 298 (1978) 921–927.
[43] A.H. Filipovich, J.V. Stone, S.C. Tomany, M. Ireland, C. Kollman, C.J. Pelz, J.T. Casper, M.J. Cowan, J.R. Edwards, A. Fasth, R.P. Gale, A. Junker, N.R. Kamani, B.J. Loechelt, D.W. Pietryga, O. Ringdén, M. Vowels, J. Hegland, A.V. Williams, J.P. Klein, K.A. Sobocinski, P.A. Rowlings, M.M. Horowitz, Impact of donor type on outcome of bone marrow transplantation for Wiskott-Aldrich syndrome: collaborative study of the International Bone Marrow Transplant Registry and the National Marrow Donor Program, Blood 97 (2001) 1598–1603.
[44] H. Ozsahin, M. Cavazzana-Calvo, L.D. Notarangelo, A. Schulz, A.J. Thrasher, E. Mazzolari, M.A. Slatter, F. Le Deist, S. Blanche, P. Veys, A. Fasth, R. Bredius, P. Sedlacek, N. Wulffraat, J. Ortega, C. Heilmann, A. O'Meara, J. Wachowiak, K. Kalwak, S. Matthes-Martin, T. Gungor, A. Ikinciogullari, P. Landais, A.J. Cant, W. Friedrich, A. Fischer, Long-term outcome following hematopoietic stem-cell transplantation in Wiskott-Aldrich syndrome: collaborative study of the European Society for Immunodeficiencies and European Group for Blood and Marrow Transplantation, Blood 111 (2008) 439–445.
[45] J. Kraunus, D.H. Schaumann, J. Meyer, U. Modlich, B. Fehse, G. Brandenburg, D. von Laer, H. Klump, A. Schambach, J. Bohne, C. Baum, Self-inactivating retroviral vectors with improved RNA processing, Gene Ther. 11 (2004) 1568–1578.
[46] D. Zychlinski, A. Schambach, U. Modlich, T. Maetzig, J. Meyer, E. Grassman, A. Mishra, C. Baum, Physiological promoters reduce the genotoxic risk of integrating gene vectors, Mol. Ther. 16 (2008) 718–725.
[47] F. Mazurier, O.I. Gan, J.L. McKenzie, M. Doedens, J.E. Dick, Lentivector-mediated clonal tracking reveals intrinsic heterogeneity in the human hematopoietic stem cell compartment and culture-induced stem cell impairment, Blood 103 (2004) 545–552.
[48] D.R. Kennedy, K. McLellan, P.F. Moore, P.S. Henthorn, P.J. Felsburg, Effect of ex vivo culture of CD34+bone marrow cells on immune reconstitution of XSCID dogs following allogeneic bone marrow transplantation, Biol. Blood Marrow Transplant. 15 (2009) 662–670.
[49] E. Montini, D. Cesana, M. Schmidt, F. Sanvito, C.C. Bartholomae, M. Ranzani, F. Benedicenti, L.S. Sergi, A. Ambrosi, M. Ponzoni, C. Doglioni, C. Di Serio, C. von Kalle, L. Naldini, 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. 119 (2009) 964–975.
[50] G. Maruggi, S. Porcellini, G. Facchini, S.K. Perna, C. Cattoglio, D. Sartori, A. Ambrosi, A. Schambach, C. Baum, C. Bonini, C. Bovolenta, F. Mavilio, A. Recchia, Transcriptional enhancers induce insertional gene deregulation independently from the vector type and design, Mol. Ther. 17 (2009) 851–856.
[51] M.P. Brown, D.J. Topham, M.Y. Sangster, J. Zhao, K.J. Flynn, S.L. Surman, D.L. Woodland, P.C. Doherty, A.G. Farr, P.K. Pattengale, M.K. Brenner, Thymic lymphoproliferative disease after successful correction of CD40 ligand deficiency by gene transfer in mice, Nat. Med. 4 (1998) 1253–1260.
[52] A.J. Hartlerode, R. Scully, Mechanisms of double-strand break repair in somatic mammalian cells, Biochem. J. 423 (2009) 157–168.
[53] Y.G. Kim, J. Cha, S. Chandrasegaran, Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 1156–1160.
[54] B.L. Stoddard, Homing endonuclease structure and function, Q. Rev. Biophys. 38 (2005) 49–95.
[55] J. Jarjour, H. West-Foyle, M.T. Certo, C.G. Hubert, L. Doyle, M.M. Getz, B.L. Stoddard, A.M. Scharenberg, High-resolution profiling of homing endonuclease binding and catalytic specificity using yeast surface display, Nucleic Acids Res. 37 (2009) 6871–6880.
[56] A. Lombardo, P. Genovese, C.M. Beausejour, S. Colleoni, Y.L. Lee, K.A. Kim, D. Ando, F.D. Urnov, C. Galli, P.D. Gregory, M.C. Holmes, L. Naldini, Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery, Nat. Biotechnol. 25 (2007) 1298–1306.