

New frontiers in the therapy of primary immunodeficiency: From gene addition to gene editing



Donald B. Kohn, MD,^{a,b,c,d} and Caroline Y. Kuo, MD^a Los Angeles, Calif

The most severe primary immune deficiency diseases (PIDs) have been successfully treated with allogeneic hematopoietic stem cell transplantation for more than 4 decades. However, such transplantations have the best outcomes when there is a well-matched donor available because immune complications, such as graft-versus-host disease, are greater without a matched sibling donor. Gene therapy has been developed as a method to perform autologous transplantations of a patient's own stem cells that are genetically corrected. Through an iterative bench-to-bedside-and-back process, methods to efficiently add new copies of the relevant gene to hematopoietic stem cells have led to safe and effective treatments for several PIDs, including forms of severe combined immune deficiency, Wiskott-Aldrich syndrome, and chronic granulomatous disease. New methods for gene editing might allow additional PIDs to be treated by gene therapy because they will allow the endogenous gene to be repaired and expressed under its native regulatory elements, which are essential for genes involved in cell processes of signaling, activation, and proliferation. Gene therapy is providing exciting new treatment options for patients with PIDs, and advances are sure to continue. (J Allergy Clin Immunol 2017;139:726-32.)

Key words: Hematopoietic stem cell transplantation, gammaretroviral vector, lentiviral vector, gene editing, site-specific endonuclease, zinc finger nuclease, CRISPR/Cas9

Gene therapy has developed from an attractive but unrealized concept to a first licensed medicine over the past few decades. Much of the work on gene therapy for primary immune deficiency

Abbreviations used

ADA-SCID:	Adenosine deaminase-deficient severe combined immunodeficiency
CGD:	Chronic granulomatous disease
CRISPR:	Clustered regularly interspaced short palindromic repeat
CVID:	Common variable immune deficiency
HSC:	Hematopoietic stem cell
HSCT:	Hematopoietic stem cell transplantation
IPEX:	Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome
LV:	Lentiviral vector
PID:	Primary immune deficiency disease
SCID:	Severe combined immune deficiency
SIN:	Self-inactivating
STAT:	Signal transducer and activator of transcription
TALEN:	Transcription activator-like effector nuclease
TIGET:	San Raffaele Telethon Institute for Gene Therapy
WAS:	Wiskott-Aldrich syndrome
X-CGD:	X-linked chronic granulomatous disease
X-HIM:	X-linked hyper-IgM syndrome
XLA:	X-linked agammaglobulinemia
X-SCID:	X-linked severe combined immunodeficiency
ZFN:	Zinc finger nuclease

diseases (PIDs) has been based around the application of hematopoietic stem cell transplantation (HSCT) as a potentially lifelong curative therapy. Because HSCT with bone marrow transplantation was first successfully performed for patients with PIDs with severe combined immune deficiency (SCID) in the late 1960s,¹ the power of replacing a patient's hematopoietic stem cells (HSCs) bearing a disease-causing mutation that disables the immune system with HSCs from a healthy donor has advanced greatly.

HSCT has been successfully applied to patients with many of the most severe PIDs, including SCID, Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD), X-linked hyper-IgM syndrome (X-HIM), leukocyte adhesion deficiency, and others. The success of HSCT has relied on identification of a well-matched stem cell donor, ideally an HLA-matched sibling or family member. For SCID, such matched sources of HSCs can provide a graft that is accepted without conditioning to eliminate the patient's endogenous stem cells or immune system. It was learned that for patients with less severe cell-mediated immune defects (eg, WAS and CGD), it was necessary to apply some regimen of cytoreductive conditioning to "make space" for donor HSCs to engraft and often immunosuppressive drugs to prevent residual immunity from rejecting the donor's HSCs.² Increased pretreatment conditioning increases the risks of allogeneic

From ^athe Department of Pediatrics; ^bthe Department of Microbiology, Immunology & Molecular Genetics; ^cthe Eli & Edythe Broad Center of Stem Cell Research & Regenerative Medicine; and ^dJonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles.

Disclosure of potential conflict of interest: D. B. Kohn is a member of the scientific advisory boards for Kite Pharma and Orchard Therapeutics; has received grants from the National Institutes of Health, the California Institute for Regenerative Medicine, and the Doris Duke Charitable Foundation; has patents through the University of California-Los Angeles; and has received royalties from patents licensed from UCLA to Orchard Therapeutics and BioMarin. C. Y. Kuo declares that she has no relevant conflicts of interest.

Received for publication December 19, 2016; revised January 20, 2017; accepted for publication January 23, 2017.

Corresponding author: Donald B. Kohn, MD, 3165 Terasaki Life Science Bldg, 610 Charles E Young Dr East, Los Angeles, CA 90095. E-mail: dkohn1@mednet.ucla.edu.

Ⓜ The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/\$36.00

© 2017 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2017.01.007>

Terms in boldface and italics are defined in the glossary on page 727.

HSCT because of direct toxic effects of the conditioning agents (often cytotoxic chemotherapy drugs), immune suppression in patients with pre-existing immune defects, and potential burdens of chronic infections.

Because the majority of patients with PIDs will not have an eligible HLA-matched family donor, techniques were developed to use HSCs from less well-matched donors, either unrelated donors identified through increasingly large registries or half-matched parental donors. These mismatched transplants carry higher risks of either graft rejection or the converse, graft-versus-host disease, in which donor T cells attack the patient's body. Therefore manipulation of the donor graft, such as T-cell depletion, or more potent immune suppression might be needed around the time of transplantation, with attendant morbidity. As experience with these more challenging transplantations has increased, outcomes have steadily improved.

Nevertheless, alternative approaches that might be safer with the same prospect of benefit are needed. The key concept of gene therapy for PIDs has been to provide an autologous HSCT option, using the patient's own HSCs that are corrected *ex vivo* by either adding a normal copy of the responsible disease-related gene or, in the near future, correcting the endogenous defective gene *in situ* in the HSCs (Fig 1). The key technical challenge has been to effectively and nontoxically introduce the normal gene into the stem cells without causing them to lose their stem cell capacity for lifelong multilineage blood cell production. Over 2 to 3 decades, such techniques have been developed and used in clinical trials for a growing number of diseases (Table I).

TECHNICAL ADVANCES ENABLING EFFECTIVE GENE THERAPY OF PIDs

Successes in gene therapy have been due to 3 sets of advances: better vectors, better HSC processing methods, and better conditioning regimens. In the mid-1980s, murine retroviruses were developed that could transfer a foreign gene into mammalian cells (Fig 2).³ Several studies demonstrated their ability to introduce their gene into murine HSCs, which could be transplanted and give rise to blood cells of both myeloid and lymphoid lineages containing and expressing the new gene.^{4,5} Initial attempts to use these vectors with human HSCs were much less effective, but improvements in vector production methods to yield higher vector titers and changes in the viral envelope protein used to coat the virus and target it to cellular receptors led to higher levels of gene transfer to human HSC. Simultaneously, new hematopoietic growth factors were identified that could stimulate activation and proliferation of human HSCs, which greatly increased their ability to take up the vectors.⁶ Additionally, it was learned that having an extracellular matrix protein, such as fibronectin or recombinant fragments thereof, could increase viral uptake by stem cells and also better preserve their activity during the 2 to 4 days of *ex vivo* culture.⁷ Finally, use of relatively low doses of the myelotoxic drug busulfan was shown to greatly enhance re-engraftment of the *ex vivo*-modified HSCs by creating space in the bone marrow niche.^{8,9}

Bringing these advances together in the late 1990s, several investigators were able to provide significant benefit to patients with adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID) and X-linked severe combined

GLOSSARY

ADENO-ASSOCIATED VIRUS (AAV): A small virus that commonly infects human subjects and primates but does not often cause disease. AAV's lack of pathogenicity makes it an ideal reagent for gene editing.

BRUTON TYROSINE KINASE (BTK): A kinase enzyme that plays a critical role as a signaling molecule in B-cell maturation and mast cell activation through the high-affinity IgE receptor. The gene defective in patients with XLA encodes BTK.

CCR5: A chemokine receptor on the surface of white blood cells, including T cells. HIV often binds CCR5 before entry into the cell.

CD45: Also called leukocyte common antigen, a receptor-type protein that is a member of the tyrosine phosphatase protein family expressed on most hematopoietic cells and known to be signaling molecules involved in activation and differentiation.

CD154 (CD40 LIGAND): A protein of the TNF superfamily that is expressed on activated T cells and binds to CD40 on antigen-presenting cells. Binding of CD154 to CD40 leads to activation of the antigen-presenting cells, inducing a variety of immune responses, particularly immunoglobulin class switch recombination.

c-Kit: A receptor tyrosine kinase protein that is important in cell survival, proliferation, and differentiation of hematopoietic stem cells.

CLUSTERED REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS/ CRISPR-ASSOCIATED PROTEIN-9 NUCLEASE (CRISPR/ Cas9): An engineered gene-editing technique that uses the bacterial enzyme Cas9 to cleave DNA at specific sites through complexing with a short guide RNA specific for a target DNA sequence.

FORKHEAD BOX P3 (FOXP3): The gene encoding the transcription factor FOXP3, which is responsible for the development and inhibitory

function of regulatory T cells. The FOXP3 protein is a key factor in maintaining homeostasis of the immune system through the suppressive effect of regulatory T cells and also regulates the expansion of conventional T cells.

GAMMARETROVIRAL VECTORS: Viruses of the family Retroviridae, which are used as tools to transfer nucleic acids by using reverse transcriptase to insert its own RNA into host DNA.

PROTO-ONCOGENE: A normal gene that can become an oncogene caused by a mutation or increased expression. Proto-oncogenes encode proteins associated with cell growth and differentiation and cell death.

SERPINE1: A gene that encodes the human C1 inhibitor protein, which is a type of serine protease inhibitor (Serpin) that blocks the activity of certain proteins in the blood, controlling inflammation.

TRANSCRIPTION ACTIVATOR-LIKE EFFECTOR NUCLEASES (TALENs): Similar to ZFNs, TALENs are engineered restriction enzymes created by the fusion a TAL effector DNA-binding domain to a DNA cleavage domain. However, unlike ZFNs, which recognize triplet nucleotides, TALENs can recognize single nucleotides, which makes interactions between TALEN DNA-binding domains and its target nucleotide easier to engineer.

ZINC FINGER NUCLEASES (ZFN): Engineered restriction enzymes that are composed of a zinc finger DNA-binding domain fused to a DNA-cleavage domain. ZFN technology generates precise targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations, creating cells with specific gene deletions, integrations, or modifications.

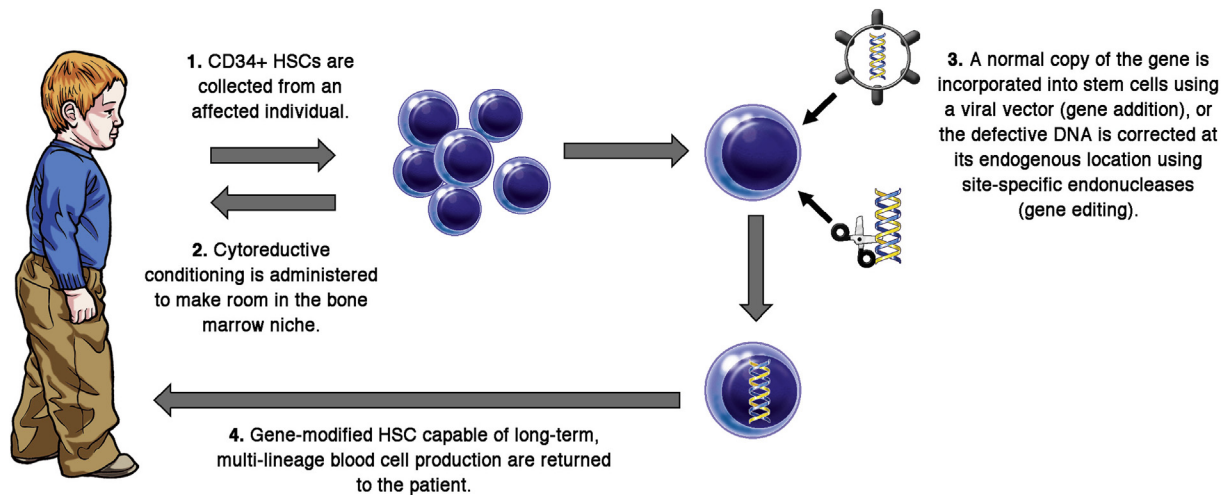


FIG 1. Clinical schema of gene therapy for PIDs.

TABLE I. Diseases treated with gene therapy using HSCs in clinical trials

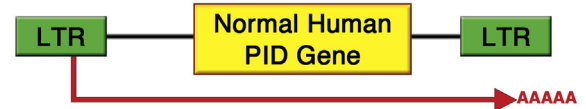
PIDs
ADA-SCID
X-SCID
WAS
X-CGD
Leukocyte adhesion deficiency (LAD)
Lysosomal storage and metabolic disorders
X-linked adrenoleukodystrophy (X-ALD)
Metachromatic leukodystrophy (MLD)
Hemoglobinopathies
β-Thalassemia
Sickle cell disease
Stem cell defects
Fanconi anemia

immunodeficiency (X-SCID) by using gene therapy with patients' autologous bone marrow HSCs.⁸⁻¹⁹ Similar studies for WAS and X-linked chronic granulomatous disease (X-CGD) followed and also led to restoration of the defective immune cell function.²⁰⁻²³

However, severe complications occurred in many of these patients, with leukemia developing a few years after gene therapy.²⁴⁻²⁹ The retroviral vectors used were randomly inserted into the chromosomal DNA of the target stem cells, and the potent enhancer elements they carried to drive expression of the therapeutic gene could transactivate expression of nearby cellular *proto-oncogenes* (*LMO2* and *MECOM*). This eventually led to clonal outgrowth of transformed cells. Although most of the patients were successfully treated for leukemia, this was an unacceptable level of toxicity.

Fortunately, the remedy was already in hand. A new generation of gene delivery vectors, derived from the lentivirus class of retroviruses, had been developed that had markedly better safety profiles. Lentiviral vectors (LVs) could be produced in a way in which the enhancer elements were self-inactivating (so-called SIN vectors) so they had minimal, if any, ability to turn on cellular genes near their integration sites (Fig 2).^{30,31} Preclinical studies in murine cells showed SIN LVs to have essentially no detectable

Gammaretroviral Vector



"SIN" Lentiviral Vector

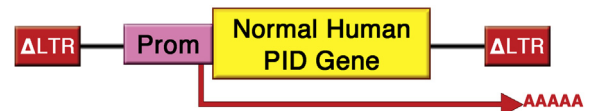


FIG 2. Gene delivery vectors used for gene therapy of PIDs. *Upper*, Gammaretroviral vectors have viral long terminal repeats (*LTR*) at each end, with viral enhancers and promoters that drive transcription and termination/polyadenylation of a normal copy of the relevant human gene involved in the inherited PIDs. The mRNA produced from the vector is shown as a red arrow, including the polyA tail. *Lower*, The SIN LV has an LTR with the enhancers deleted (Δ) and an internal promoter (*Prom*) to drive transcription of the human PID gene. In current clinical trials LVs being used for ADA-SCID and X-SCID are using the human elongation factor $\alpha 1$ gene short promoter (EFS) to drive the *ADA* and *IL2RG* genes, respectively; the WASP gene endogenous promoter to drive a WASP gene; and a chimeric myeloid promoter to drive the gp91phox gene for X-CGD.

transformative activity, whereas that of murine *gammaretroviral vectors* was readily demonstrable.³²⁻³⁶ Thus most trials currently use SIN LVs, and ongoing trials for ADA-SCID, X-SCID, WAS, and X-CGD have shown effective immune reconstitution and no vector-related complications to date, with more than 100 patients treated since 2009, when the earliest trial with an LV inserted into HSCs (for X-linked adrenoleukodystrophy) was reported.³⁷⁻⁴⁰ Additionally, one study used a SIN gammaretroviral vector to treat X-SCID, with no complications of clonal expansion and good immune reconstitution in the majority of patients.⁴¹ Based on the elapsed time since they were reported to be absent of clonal proliferations in 2014 and the subsequent lack of occurrences, they are all now beyond the period during which all of the cases of leukoproliferation occurred in the earlier X-SCID trials (A. Thrasher/D. A. Williams, personal communication).

One exception to the complications with murine gammaretroviral vectors has been in the setting of ADA-SCID. More than

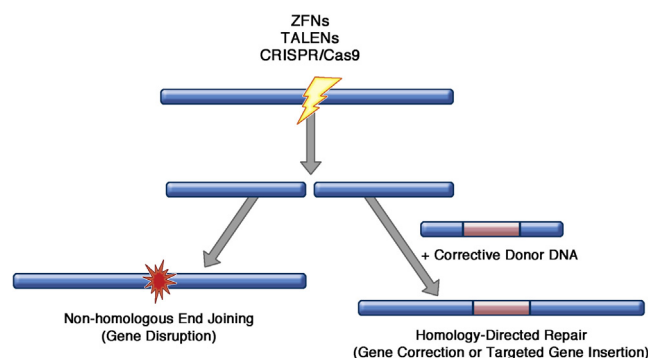


FIG 3. Gene editing using site-specific endonucleases. Site-specific endonucleases (ZFNs, TALENs, or CRISPR/Cas9) can be used to create targeted double-stranded DNA breaks. These DNA breaks can be repaired by means of nonhomologous end-joining, which introduces small insertions and deletions and can be used for gene knockout strategies, as used in the CCR5 gene therapy trials for HIV. If a corrective donor DNA sequence is also provided to the cell, it can be used for homology-directed repair of the double-stranded DNA break, incorporating the therapeutic gene sequence and maintaining its expression under its endogenous regulatory elements.

40 patients with ADA-SCID have been safely treated with no leukemia-like complications using gammaretroviral vectors since the landmark studies from the San Raffaele Telethon Institute for Gene Therapy (TIGET), Milan, Italy, began in the late 1990s, followed by studies at University College London/Great Ormond Street Hospital and the University of California, Los Angeles.^{9,11,13,14} The work from TIGET led to approval by the European Medicines Agency of their gammaretroviral vector gene therapy for ADA-SCID, which is marketed by GlaxoSmithKline (Research Triangle Park, NC) as Strimvelis. It is only the second gene therapy product to receive licensure in the European Union (with none in the United States or Canada to date). Why the gammaretroviral vectors have not had clinical safety problems in contrast to similar vectors for other PIDs remains unknown. Both the TIGET and University of California, Los Angeles groups have demonstrated vector integrations near some of the same genes implicated in leukoproliferation in patients with other diseases, but there have been no clinical manifestations thus far.⁴²

It should be emphasized that in all of these gene therapy trials, there has been no need to use immunosuppressive drugs (eg, cyclophosphamide, fludarabine, ATG, cyclosporine, and corticosteroids), and there have been no problems with graft rejection or graft-versus-host disease. The amounts of pretransplantation conditioning used for the non-SCID disorders (WAS and CGD) were higher (8-12 mg/kg busulfan) than those used for ADA-SCID (4 mg/kg) and X-SCID (0-8 mg/kg) because of the perceived need for greater cytoablation for higher engraftment of the corrected stem cells to correct all blood cell lineages and not just lymphocytes, as is needed for patients with SCID. Although the dosages of these potent drugs are significant and have some risk, the results to date have been gratifyingly good, with only the expected marrow suppression and some mucositis and no severe transplant-related complications.

FUTURE APPROACHES AND PROSPECTS FOR GENE THERAPY OF PIDs

One of the drawbacks of gene therapy is that each disease genotype entails a separate drug development project to develop the vector for that specific gene, perform preclinical activity and

toxicity studies, and so on. In contrast, in allogeneic HSCT donor cells have all of the corrective genes, and it is more of a “one size fits all” approach. Nevertheless, there are ongoing projects at many centers to develop gene therapy for additional PIDs. Other forms of SCID, such as Artemis and recombination-activating genes 1 and 2 deficiency, autosomal recessive forms of CGD (p47 and p67), and other PIDs (leukocyte adhesion deficiency and hereditary lymphohistiocytosis) are under preclinical study with the goal of advancing to early-phase clinical trials. Thus these gene therapy approaches of gene addition will compete with the always improving methods of allogeneic HSCT to provide the best treatments for many of these diseases.

However, for other PIDs, gene-addition therapies are not currently applicable. Common variable immune deficiency (CVID), perhaps the most prevalent PID, might likely be caused by one of several genes, only a few of which are currently recognized. Each will again entail a separate development process, and the efficacy and safety will need to be high enough to outweigh the results obtained currently with best medical therapy. Gain-of-function mutations, such as in signal transducer and activator of transcription 1 (*STAT1*) and *STAT3*, where the mutant allele can override proper expression of a new normal copy of the gene, poses additional challenges to traditional forms of gene therapy.

Additionally, genes that underlie some PIDs might not be amenable to the approach of gene addition by using a viral vector. Genes involved in cell activation, intracellular signaling, and other processes likely require precise temporal and physiologic control of their expression. For example, after the CD40 ligand gene was identified as a cause of X-HIM, studies were done to add that gene to bone marrow HSCs from a gene knockout mouse model.⁴³ Although initial immune restoration was achieved with class-switching of B cells, many of the mice had lymphoproliferative complications from constitutive and unregulated expression of CD40 ligand. Other genes involved in PIDs that might similarly require precise regulation are *Bruton tyrosine kinase (BTK)*, which is involved in X-linked agammaglobulinemia; forkhead box P3 (*FOXP3*), which causes immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX); and some of the B-cell growth factors or their receptors that might be involved in some cases of CVID (eg, transmembrane activator and CAML interactor and B cell-activating factor receptor). Efforts to derive vectors that display the precise regulation pattern of the endogenous gene have often been unsuccessful because it can be difficult to identify all of the DNA regulatory sequences that might surround and regulate these genes to include in the relatively small genome size of a vector.

GENE EDITING TO CORRECT THE GENES CAUSING PIDs

Once again, the answer is close at hand. New methods have been developed in the past few years for gene correction. If a disease-related gene could be repaired in its native chromosomal site, it should retain the normal expression pattern and restore the needed genetic function. A real revolution of scientific advances has developed methods for gene editing that can either disrupt and turn off a troublesome gene (eg, dominant *STAT2*), repair a gene mutation (eg, in *BTK*), or insert a new copy of the gene directly into the defective gene itself to use the gene expression regulatory elements to control expression of the inserted gene (eg, CD154). The critical component of this revolution in gene editing has been

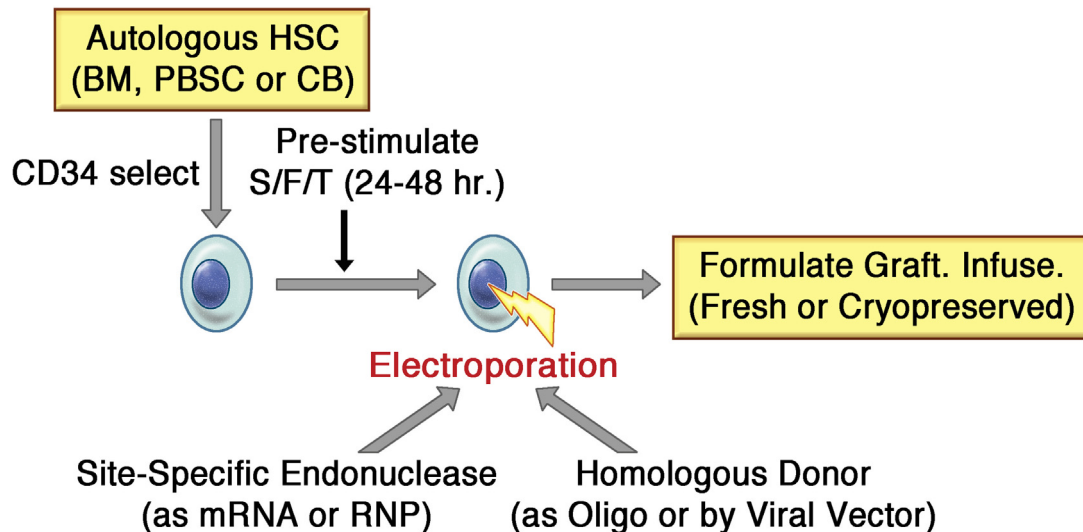


FIG 4. Site-specific gene editing of autologous HSCs for gene therapy of PIDs. Autologous HSCs can be obtained from bone marrow (BM), mobilized peripheral blood stem cells (PBSC), or umbilical cord blood (CB). Usually the HSCs are enriched through CD34 immunoselection. HSCs are prestimulated to improve gene modification by means of culture for 24 to 48 hours with a combination of recombinant hematopoietic growth factors, such as c-Kit ligand/Flt-3 ligand and thrombopoietin (S/F/T). Cells are then treated with electroporation with the site-specific endonuclease (ZFN, homing endonuclease, TALEN, or CRISPR) to introduce a double-stranded break at the target gene and delivered as either *in vitro* transcribed mRNA or preformed ribonucleoprotein (RNP) containing the Cas9 protein and a short-guide RNA for CRISPR-mediated processes. The homologous donor containing corrective sequences can be provided either as an oligonucleotide (Oligo) that is coelectroporated with the endonuclease or through a viral vector (eg, adeno-associated virus or integration-defective lentiviral vector). After electroporation, gene-modified HSCs are formulated for intravenous administration and can either be infused into the patient fresh or after cryopreservation and thawing.

the recognition that such gene-editing events can be greatly enhanced by introducing a precisely located break in the chromosomal DNA near the site of intended gene editing.⁴⁴ A succession of designer site-specific endonucleases have been developed, including homing endonucleases, *zinc finger nucleases* (ZFN), *transcription activator–like effector nucleases* (TALENs), and, more importantly, *clustered regularly interspaced short palindromic repeat* (CRISPR/Cas9). Each of these systems can be engineered to recognize and introduce a DNA break at essentially one site out of the entire genome. If the DNA break is left to be resolved by the cell, it is often reannealed through a process called nonhomologous end-joining that often removes or adds a few base pairs at the break site and leads to disruption of the gene's reading frame and gene silencing. However, if the cell is provided at the same time with copies of the target sequence as single-stranded or double-stranded DNA carrying the corrective DNA sequence, it might use the introduced information to repair the break by using homology-directed repair, “knocking in” the corrective sequence and repairing the pathogenic mutation (Fig 3). This process of homology-directed repair can even be used to introduce new gene cassettes at a precise target site, such as a full-length normal copy of the defective gene just downstream from the gene promoter sequences, to maintain the gene's normal expression patterns.

These methods for gene editing have entered the clinic, with the first study using ZFNs to disrupt the HIV *CCR5* coreceptor in peripheral blood T cells from patients infected by HIV, rendering the cells relatively resistant to HIV infection.⁴⁵ Other studies nearing clinical trials will seek to disrupt the *CCR5* gene in HSCs of HIV-infected patients or disrupt the gene encoding a protein (*Bcl11a*)

that represses expression of fetal globin to turn on the fetal hemoglobin as a way to treat β -thalassemia and sickle cell disease.

Gene correction in HSCs for PIDs has been demonstrated in preclinical studies for X-SCID, ADA-SCID, X-CGD, X-HIM, and others.^{46–48} The methods are reaching levels of efficacy and nontoxicity to HSCs to lead to clinical efficacy (Fig 4). Gene-editing approaches might allow a much wider range of PIDs to be treated with gene therapy and autologous transplantation, including X-linked agammaglobulinemia (XLA), X-HIM, IPEX, CVID, and others.

In addition to all of the cell-based PIDs discussed above, there are other PIDs that are caused by defects in blood proteins, such as hereditary angioedema. A recent report described studies in a mouse model of hereditary angioedema in which a different viral vector, *adeno-associated virus*, was used to introduce a normal *SERPINE1* gene encoding the C1 inhibitor into cells of the liver that could then serve as a factory to produce the protein and release it into the bloodstream.⁴⁹ This approach of a protein factory has also been shown to be effective for ADA-SCID. In the mouse model an LV carrying a normal human ADA gene was injected intravenously, targeted the liver, and served as a source of ADA sufficient to sustain survival and correct immunity. Similar approaches are under active study for non-PID protein disorders, such as hemophilia, antitrypsin inhibitor deficiency, and others.

REMAINING CHALLENGES FOR GENE THERAPY FOR PIDs

Of course, there are challenges that remain to be fully overcome. Production of gene-addition vectors or gene-editing

reagents has been on a relatively small scale for pilot studies and needs to be advanced to pharmaceutical production methods at sufficient scale to treat many patients. Both gene addition with SIN LVs and gene editing with the various endonucleases can still have some risk of genotoxicity if they disrupt important genes by means of either insertion or off-target cutting.

The conditioning regimens used to date have been based on chemotherapy regimens. Although these have not shown significant toxicity in the short term, they can have some late effects, even at the reduced dosages used. Once again, progress has been made in this essential area through the development of methods to “make space” for transplanted gene-corrected HSCs using mAbs to proteins on HSCs, such as *c-Kit* or *CD45*.⁵⁰⁻⁵² If effective, these new conditioning agents could greatly increase safety and allow applications of gene therapy to the somewhat milder PIDs, such as XLA.

Although current approaches of either gene addition or gene editing are done *ex vivo* with harvested bone marrow or mobilized stem cells, it can be advantageous, when possible, to deliver the needed gene or gene-editing reagents *in vivo* and repair the HSCs while remaining in their niches. Eventually, use of pluripotent stem cells, such as induced pluripotent stem cells, might allow autologous cell grafts to be produced, gene corrected, expanded, and used for transplantation, with a much more abundant source of stem cells providing better immune reconstitution.⁵³

It is an exciting time for science and a good time for patients because these new treatments using gene therapy can provide safer and more effective approaches that avoid the complications of allogeneic HSCT. Of course, it will take several years of comparative analyses to determine which approaches are best, but the existence of these various treatments for diseases that were once often fatal is indeed gratifying.

Key concepts

- Severe PIDs can be treated by means of transplantation of HSCs from a healthy matched donor, but risks of immunologic complications exist and are heightened when the transplant is from an unrelated donor or haploidentical donor.
- Gene therapy for PIDs allows an autologous transplantation in which the patient's own HSCs are corrected and transplanted, essentially eliminating immunologic complications.
- HSCs can be corrected by addition of a normal copy of the relevant gene using an integrating retroviral vector or LV.
- Initial studies with retroviral vectors showed clinical efficacy, but some had leukoproliferative complications from the vectors (this has not been seen for ADA-SCID).
- Current trials with safer LVs lacking strong enhancers have shown efficacy for ADA-SCID, X-SCID, WAS, and CGD, with no vector-related complications.
- Recently, methods have been developed for gene editing that could allow the disease-causing mutation to be corrected *in situ* within a patient's HSCs. This approach might be of particular value for disorders in which the relevant PID-causing gene requires precise regulation of expression, such as CD40 ligand for X-HIM, BTK for XLA, and FOXP3 for IPEX.

Therapeutic implications

- Gene therapy provides a new therapeutic option for the treatment of patients with severe PIDs that respond to allogeneic HSCT.

REFERENCES

1. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet* 1968;2:1366-9.
2. Parkman R, Rapoport J, Geha R, Belli J, Cassady R, Levey R, et al. Complete correction of the Wiskott-Aldrich syndrome by allogeneic bone-marrow transplantation. *N Engl J Med* 1978;298:921-7.
3. Miller AD, Rosman GJ. Improved retroviral vectors for gene transfer and expression. *Biotechniques* 1989;7:980-2, 984-6, 989-90.
4. Williams DA, Lemischka IR, Nathan DG, Mulligan RC. Introduction of new genetic material into pluripotent haematopoietic stem cells of the mouse. *Nature* 1984;310:476-80.
5. Dick JE, Magli MC, Huszar D, Phillips RA, Bernstein A. Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/Wv mice. *Cell* 1985;42:71-9.
6. Nolte JA, Crooks GM, Overell RW, Williams DE, Kohn DB. Retroviral vector-mediated gene transfer into primitive human hematopoietic progenitor cells: effects of mast cell growth factor (MGF) combined with other cytokines. *Exp Hematol* 1992;20:1065-71.
7. Moritz T, Patel VP, Williams DA. Bone marrow extracellular matrix molecules improve gene transfer into human hematopoietic cells via retroviral vectors. *J Clin Invest* 1994;93:1451-7.
8. 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.
9. Candotti F, Shaw KL, Muul L, Carbonaro D, Sokolic R, Choi C, et al. Gene therapy for adenosine deaminase-deficient severe combined immune deficiency: clinical comparison of retroviral vectors and treatment plans. *Blood* 2012;120:3635-46.
10. Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N Engl J Med* 2009;360:447-58.
11. Cicalese MP, Ferrua F, Castagnaro L, Pajno R, Barzaghi F, Giannelli S, et al. Update on the safety and efficacy of retroviral gene therapy for immunodeficiency due to adenosine deaminase deficiency. *Blood* 2016;128:45-54.
12. Gaspar HB, Björkregren 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.
13. Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Zhang F, Adams S, et al. Hematopoietic stem cell gene therapy for adenosine deaminase-deficient severe combined immunodeficiency leads to long-term immunological recovery and metabolic correction. *Sci Transl Med* 2011;3:97ra80.
14. Candotti F, Shaw KL, Muul L, Carbonaro D, Sokolic R, Choi C, et al. Phase II clinical trial of gene therapy for adenosine deaminase-deficient severe combined immune deficiency (ADA-SCID). *Mol Ther* 2014;22(suppl 1):S107.
15. Cavazzana-Calvo M. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000;288:669-72.
16. Hacein-Bey-Abina S, Le Deist F, Carlier F, Bouneaud C, Hue C, De Villartay JP, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J Med* 2002;346:1185-93.
17. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2010;363:355-64.
18. Gaspar HB, Parsley KL, Howe S, King D, Gilmour KC, Sinclair J, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. *Lancet* 2004;364:2181-7.
19. Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Adams S, Howe SJ, et al. Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency. *Sci Transl Med* 2011;3:97ra79.

20. Boztug K, Schmidt M, Schwarzer A, Banerjee PP, Díez IA, Dewey RA, et al. Stem-cell gene therapy for the Wiskott-Aldrich syndrome. *N Engl J Med* 2010; 363:1918-27.
21. Kang EM, Choi U, Theobald N, Linton G, Long Priel DA, Kuhns D, et al. Retroviral gene therapy for X-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils. *Blood* 2010;115:783-91.
22. Kang HJ, Bartholomae CC, Paruzynski A, Arens A, Kim S, Yu SS, et al. Retroviral gene therapy for X-linked chronic granulomatous disease: results from phase I/II trial. *Mol Ther* 2011;19:2092-101.
23. 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-EV11, PRDM16 or SETBP1. *Nat Med* 2006;12: 401-9.
24. Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E, et al. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2003;348:255-6.
25. Hacein-Bey-Abina S. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003;302:415-9.
26. Fischer A, Abina SH, Thrasher A, von Kalle C, Cavazzana-Calvo M. LMO2 and gene therapy for severe combined immunodeficiency. *N Engl J Med* 2004;350: 2526-7.
27. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kemp-ski 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.
28. Stein S, Ott MG, Schultze-Strasser S, Jauch A, Burwinkel B, Kinner A, et al. Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. *Nat Med* 2010;16:198-204.
29. Braun CJ, Boztug K, Paruzynski A, Witzel M, Schwarzer A, Rothe M, et al. Gene therapy for Wiskott-Aldrich syndrome—long-term efficacy and genotoxicity. *Sci Transl Med* 2014;6:227ra33.
30. Naldini L, Blömer U, Gally P, Ory D, Mulligan R, Gage FH, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* 1996;272:263-7.
31. Naldini L, Trono D, Verma IM. Lentiviral vectors, two decades later. *Science* 2016;353:1101-2.
32. Modlich U, Navarro S, Zychlinski D, Maetzig T, Knoess S, Brugman MH, et al. Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. *Mol Ther* 2009;17:1919-28.
33. Carbonaro DA, Zhang L, Jin X, Montiel-Equihua C, Geiger S, Carmo M, et al. Preclinical demonstration of lentiviral vector-mediated correction of immunological and metabolic abnormalities in models of adenosine deaminase deficiency. *Mol Ther* 2014;22:607-22.
34. Marangoni F, Bosticardo M, Charrier S, Draghici E, Locci M, Scaramuzza S, et al. Evidence for long-term efficacy and safety of gene therapy for Wiskott-Aldrich syndrome in preclinical models. *Mol Ther* 2009; 17:1073-82.
35. Charrier S, Dupré L, Scaramuzza S, Jeanson-Leh L, Blundell MP, Danos O, et al. Lentiviral vectors targeting WASp expression to hematopoietic cells, efficiently transduce and correct cells from WAS patients. *Gene Therapy* 2007;14: 415-28.
36. Santilli G, Almaraz E, Brendel C, Choi U, Beilin C, Blundell MP, et al. Biochemical correction of X-CGD by a novel chimeric promoter regulating high levels of transgene expression in myeloid cells. *Mol Ther* 2011;19:122-32.
37. Gaspar HB, Buckland K, Carbonaro DA, Shaw K, Barman P, Davila A, et al. Immunological and metabolic correction after lentiviral vector gene therapy for ADA deficiency. *Mol Ther* 2015;23(suppl 1):S102.
38. De Ravin SS, Wu X, Moir S, Anaya-O'Brien S, Kwatema N, Littell P, et al. Lentiviral hematopoietic stem cell gene therapy for X-Linked severe combined immunodeficiency. *Sci Transl Med* 2016;8:335ra57.
39. Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. *Science* 2013;341:1233151.
40. Hacein-Bey Abina S, Gaspar HB, Blondeau J, Caccavelli L, Charrier S, Buckland K, et al. Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. *JAMA* 2015;313:1550-63.
41. Hacein-Bey-Abina S, Pai SY, Gaspar HB, Armant M, Berry CC, Blanche S, et al. A modified γ -retrovirus vector for X-linked severe combined immunodeficiency. *N Engl J Med* 2014;371:1407-17.
42. 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.
43. Brown MP, Topham DJ, Sangster MY, Zhao J, Flynn KJ, Surman SL, et al. Thymic lymphoproliferative disease after successful correction of CD40 ligand deficiency by gene transfer in mice. *Nat Med* 1998;4:1253-60.
44. Porteus MH, Baltimore D. Chimeric nucleases stimulate gene targeting in human cells. *Science* 2003;300:763.
45. Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N Engl J Med* 2014;370:901-10.
46. Lombardo A, Genovese P, Beausejour CM, Colleoni S, Lee YL, Kim KA, et al. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. *Nat Biotechnol* 2007;25:1298-306.
47. Joglekar AV, Hollis RP, Kuftinec G, Senadheera S, Chan R, Kohn DB. Integrase-defective lentiviral vectors as a delivery platform for targeted modification of adenosine deaminase locus. *Mol Ther* 2013;21:1705-17.
48. De Ravin SS, Reik A, Liu PQ, Li L, Wu X, Su L, et al. Targeted gene addition in human CD34+ hematopoietic cells for correction of X-linked chronic granulomatous disease. *Nat Biotechnol* 2016;34:424-9.
49. Pagovich OE, Qui T, Whaley AS, Russo AR, Rosenberg J, Bishnu P, et al. One-time gene therapy for hereditary angioedema. *Mol Ther* 2016;24(suppl 1):S298.
50. Czechowicz A, Kraft D, Weissman IL, Bhattacharya D. Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. *Science* 2007; 318:1296-9.
51. Chhabra A, Ring AM, Weiskopf K, Schnorr PJ, Gordon S, Le AC, et al. Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy. *Sci Transl Med* 2016;8:351ra105.
52. Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, et al. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. *Nat Biotechnol* 2016;34: 738-45.
53. Merling RK, Sweeney CL, Chu J, Bodansky A, Choi U, Priel DL, et al. An AAVS1-targeted minigene platform for correction of iPSCs from all five types of chronic granulomatous disease. *Mol Ther* 2015;23:147-57.