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*

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

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 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 [a]the Department of Pediatrics; [b]the Department of Microbiology, Immunology & Molecular Genetics; [c]the Eli & Edythe Broad Center of Stem Cell Research & Regenerative Medicine; and [d]Jonsson 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

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 reengraftment 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 the 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.

***SERPING1*:** 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.

The Editors wish to acknowledge Kristina Bielewicz, MSc, for preparing this glossary.