Gene Therapy for Primary Immunodeficiency

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Abstract
Over the past 3 decades, there has been significant progress in refining gene therapy technologies and procedures. Transduction of hematopoietic stem cells ex vivo using lentiviral vectors can now create a highly effective therapeutic product, capable of reconstituting many different immune system dysfunctions when reinfused into patients. Here, we review the key developments in the gene therapy landscape for primary immune deficiency, from an experimental therapy where clinical efficacy was marred by adverse events, to a commercialized product with enhanced safety and efficacy. We also discuss progress being made in preclinical studies for challenging disease targets and emerging gene editing technologies that are showing promising results, particularly for conditions where gene regulation is important for efficacy.

Introduction
Primary immune deficiencies (PIDs) are inherited life-threatening diseases, characterized by susceptibility to infection, increased risk of malignancy, autoimmunity, and inflammation. They arise due to abnormalities in over 300 genes governing the development or function of a range of immune subsets of both the innate and adaptive immune system.1 Globally they are rare diseases, occurring at a rate of 1:10,000 births,2 although this can be 20-fold greater in countries with a higher rate of consanguinity,3 or populations with founder mutations.4-6

Symptoms often arise in childhood and historically treatment options have been limited, focused on supportive care with hematopoietic stem cell transplant (HSCT), the only curative approach. This technique has evolved over time and the associated morbidity and mortality have dramatically reduced in some settings. However, success is still largely based on the availability of good human leukocyte antigen (HLA)-matched donor, with reduced survival in the mismatch setting arising from graft-versus-host disease (GvHD), infection, and graft rejection. Autologous gene-corrected stem cell therapy offers an attractive alternative where a suitable HLA-matched donor is unavailable, with the possibility of avoiding GvHD and often the ability to use less toxic and immunosuppressive conditioning regimens.

As the founders of the immune system, hematopoietic stem cells (HSCs) offer a relatively accessible therapeutic target through either direct bone marrow harvest or, more recently, the preferred option of leukapheresis. Following granulocyte-colony stimulating factor (G-CSF) and plerixafor-mediated mobilization from the bone marrow into the periphery, harvesting through apheresis and CD34+ cell selection, HSC can be manipulated with gene corrective tools ex vivo, before returning to the patient to engraft and restore a fully functioning system. However, it was later realized that lentiviral vectors (LVs) based on HIV-1 had a safer integration profile, largely integrating within actively transcribed genes, therefore keeping exogenous promoters contained in vectors away from regulatory regions.7,8 SIN LV vectors are now the most widely used vectors and have excellent safety track record—more than 150 primary immune deficiency patients have been treated over the past decade without developing leukemia or myelodysplasia.9-13

The burgeoning interest in gene and engineered cell therapies using viral vectors has driven the optimization of good manufacturing practice (GMP) compliant large-scale suspension
serum-free production systems and packaging cell lines. These processes reduce the amount of handling associated with adherent cell culture and reduce dependency on costly animal-derived products that carry a contamination risk and ethical concerns. Furthermore, interest has focused on the development of novel transduction enhancers that reduce the amount of high-cost virus required and cell culture media components that aim to retain HSC potency in culture. These technologies will lower the associated cost of gene therapy procedures and improve access, particularly when coupled with automated cell culture devices.

Severe combined immunodeficiencies

Severe combined immunodeficiencies represent the most lethal PIDs and occur in an estimated 1:50,000–100,000 births. They are characterized by genetic faults leading to a block in T cell development programs, combined with deficiencies in numbers or function of natural killer (NK) or B cells resulting in both cellular and humoral immune abnormalities. These conditions often present in infancy with overwhelming infection and require urgent HSCT. Outcome following transplant can be negatively impacted by active infection alongside poorly matched donor status. Many countries around the globe have recently introduced...
newborn screening programs for severe combined immunodeficiency (SCID) to improve outcome through early diagnosis. SCID caused by a lack of adenosine deaminase enzyme (ADA-SCID) accounts for 10% of SCID diagnoses. Ubiquitous and highly conserved, ADA is a key enzyme in purine metabolism, responsible for safely converting adenosine to inosine; in its absence, toxic metabolites including adenosine, 2’—deoxyadenosine, and deoxyadenosine triphosphate (dATP) accumulate, leading to profound reduction in the numbers of circulating T, B, and NK cells. Suffering with a severe lack of cellular and humoral immunity, 85% of patients present to clinic in the first year of life due to a failure to thrive and high risk of opportunistic bacterial and fungal infections, alongside systemic abnormalities affecting the lungs and skeletal system. Neurological impairments, including deafness, developmental delay, and behavioral issues, are common. In contrast to the limited treatment options for most types of SCID, ADA-deficient patients can receive enzyme replacement therapy (ERT) in the form of polyethylen glycol-conjugated bovine enzyme (PEG-ADA), which can successfully reduce the levels of metabolites and improve lymphocyte numbers. However, this is not curative, and long-term use is associated with reduced efficacy and significant cost. HSCT offers a curative therapy for patients with overall survival (OS) of 86% for those with matched sibling donors (MSD) and 81% in the matched-related donor (MRD) setting in a 106 patient cohort. However, in the mismatched donor setting survival falls to 66% for matched unrelated donors (MUD) and 43% for haploidentical donor transplants. As a monogenic disease, ADA-SCID was an attractive candidate for gene therapy. The first trials began in the 1990s, using γRV vectors to transduce and infuse T cells, umbilical cord blood cells, and bone marrow cells but failed to show long-term efficacy. Patients did not receive a preconditioning regimen, under the rationale that corrected cells would have a significant survival advantage despite continuing to receive PEG-ADA. Subsequent RV trials incorporated both myeloreductive conditioning (busulfan, melphalan) and cessation of ERT, observing restoration of lymphocyte number, reduced rates of infection, and 100% survival of over 40 treated patients. In 2016, this protocol and vector was licensed as Strimvelis (GSK2696273), the first ex vivo gene therapy product to treat a primary immune deficiency licensed in Europe.

To date, there has been no evidence of viral-mediated genotoxicity in this disease, despite evidence of integration sites near proto-oncogenes (LMO2, BCL2, CCND2) that have driven malignancy in other diseases. However, in line with safety improvements in the wider field, SIN LV vector approaches have been pursued, using the mammalian elongation factor 1 short (EFS) promoter to drive ADA expression. Murine models indicated that this vector was able to restore gene expression and restore immune function comparable to the γRV vector while demonstrating a significant reduction in transformation potential in vitro. In addition, studies in the same model revealed that while conditioning significantly improved engraftment, withholding PEG-ADA was less important, and that it may be preferable to maintain ERT to maintain cellularity in the bone marrow and reduce the period of lymphopenia post-transplant. Current trials now include pharmacokinetic (PK)-adjusted busulfan conditioning, maintained ERT until 30 days after infusion and increasingly the use of cryopreserved products allowing for more extensive testing release criteria to be completed before the product is infused (NCT02399984/NCT01380990/NCT02022696/NCT01852071) (Table 1).

The very promising results seen in trials of gene therapy for ADA-SCID in terms of long-term immune recovery and safety have led to treatment guidelines suggesting the use of gene therapy rather than allogeneic HSCT from a matched unrelated donor (European Society for Blood and Marrow Transplantation Guidelines). Unfortunately, neither gene therapy nor HSCT can improve the nonimmune-related disease manifestations seen in this condition.

X-SCID is one of the more common forms of SCID, accounting for up to 40% of cases in some populations. Mutations in the IL2RG gene lead to an absence of the common gamma chain, a vital common component of the receptors for the cytokines interleukin (IL)-2, -4, -7, -9, -15, and -21. Gamma chain deficient lymphocytes are unable to receive the signals needed to develop, leading to an absence of circulating T and NK cells and dysfunctional B cells, resulting in severe immunodeficiency and susceptibility to severe and often opportunistic infection. HSCT was previously the only curative treatment, and while this procedure can be highly successful from a geno-identical donor (OS >90%), the outcome is less favorable for patients with mismatched donors, particularly when active infection is present.

Gene therapy for X-SCID entered the clinic using a γRV vector in 1999. Clear clinical benefit was observed, with patients reconstituting functional T cells and, to a lesser extent, NK cells and reduced infections. However, 6 out of 20 patients enrolled developed T acute lymphoblastic leukemia as a result of clonal reactivation of proto-oncogenic loci and accumulated genic abnormalities including deletion of tumor suppressor genes and translocation events.

To address genotoxicity while retaining clinically efficacy, SIN γRV vectors were developed again employing the mammalian EFS promoter. T cell gene marking in treated patients was similar to the first trial, yet no severe adverse events relating to insertion mutagenesis have been recorded to date in the 9 surviving patients enrolled; the 1 death occurring due to existing viremia (NCT01410019/NCT01175239/NCT01129544). In both trials, the absence of a conditioning regimen prior to transplant contributed to suboptimal myeloid engraftment and humoral reconstitution, often requiring patients to stay on immunoglobulin therapy despite the survival advantage of corrected cells. More recently, LV vector trials for X-SCID have incorporated low-dose PK-adjusted Busulfan conditioning with the aim of improving efficacy; early results suggesting improved B cell reconstitution and normalization of immunoglobulin responses have recently been reported.

SCID can also be caused by mutations in genes encoding proteins responsible for V(D)J rearrangement of T and B cell antigen receptors, such as DNA-dependent protein kinase (DNA-PKcs), catalytic subunit. DNA ligase 4 (LIG4), recombination activating gene 1 and 2 (RAG1/2), and Artemis. The latter three have long been identified as targets for gene therapy but have faced challenges in replicating the endogenous level of gene expression that is crucial for correct function.

While an absence of RAG1 or RAG2 causes a T– B– SCID phenotype, insufficient expression leads to Omenn syndrome, immune dysregulation, and autoimmunity, as seen in some patients with hypomorphic mutations. Preclinical gene therapy studies have struggled to obtain sufficiently high levels of gene expression from vectors that are suitable for clinical use, however, following successful outcomes in a murine model, an SIN LV vector using an MND promoter construct to drive RAG1 has now been selected for translation and a trial planned for the near future, while a Ubiquitous Chromatin Opening Element (UCOE) promoter has shown promising results for RAG2.

Several groups have generated LV vectors expressing the DCLRE1C gene that encodes the Artemis protein. However, toxicity was observed when expression levels were too high. A further study utilized the endogenous Artemis promoter and found it gave optimal reconstitution in Artemis knockout (KO) mice. Some preliminary results of a trial of 5 patients (NCT03538899) using this vector have indicated the efficacy of this approach, noting the reappearance of T cell subsets along with stable gene marking in T, B, NK, and myeloid cells, allowing the patients to leave isolation.
SCIDs: paradigm for emerging therapies

For both X-SCID and ADA deficiency, the element of survival advantage in corrected lymphocytes makes these diseases attractive models for novel therapies. Techniques being tested include in vivo gene therapy, a minimally invasive technique to correct cells by direct infusion of gene transfer vectors. In vivo gene therapy has been attempted in ADA deficient mice and nonhuman primates using LV vectors, however, efficacy was limited outside the neonatal setting. In a recent study, premobilization of HSC and in vivo transduction using foamy virus vectors was corrective in a canine model of X-SCID. Foamy viruses are attractive gene transfer vectors for HSC, as they are resistant to serum inactivation, are able to transduce quiescent cells, and present a favorable integration profile; however, the safety, efficacy, and scalability of this approach remains a challenge for first-in-human studies.

Gene editing offers the potential to provide therapeutic gene expression closest to the endogenous profile by inserting corrective sequences in situ. This technique has become clinically relevant due to the development of a series of highly site-specific nucleases, including zinc-finger nucleases (ZFNs), TALE nucleases (TALENs), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein systems (CRISPR/Cas) (Figure 1C). The creation of a DNA double-strand break provides a substrate for endogenous DNA repair pathways, which can be harnessed either to KO genes or to seamlessly insert therapeutic DNA by providing a suitable donor containing sequences homologous to the cleaved ends (Figure 1D). This placement conserves many of the native regulatory motifs surrounding a gene, many of which would be too large to fit into a LV vector and are often poorly defined.

All of these technologies have now entered the clinic, although so far, none using homology-directed repair (HDR). In the absence of a homology repair template, nonhomologous end-joining (NHEJ) occurs, creating small insertions and deletions (INDELs) which lead to gene KO. The first-in-man trial, initiated in 2009, used ZFN to create autologous C-C chemokine

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ADA-SCID = adenosine deaminase severe combined immunodeficiency; CGD = chronic granulomatous disease; LAD-1 = leukocyte adhesion defect type 1; LV = lentiviral vector; NA = not available; NIH = National Institutes of Health; SOD = severe combined immunodeficiency; SIN = self-inactivating; UCLA = University of California, Los Angeles; UCSF = University of California San Francisco; WAS = Wiskott-Aldrich syndrome; γRV = gammaretrovirus; UCLA = University of California - Los Angeles; UCSF = University of California - San Francisco.

*Some patients received cryopreserved products.
receptor type 5 (CCR5) KO T cell product for patients with HIV. With 1 severe adverse event out of 12 patients, unrelated to the editing procedure, this trial showed that editing tools can be safe, particularly in T cells; TALENs and CRISPR platforms have now been used extensively in immunotherapy products such as CAR T cells. In 2018, the first gene-edited HSC trials were announced for patients with sickle cell disease (SCD) and β-thalassemia (NCT03745287/NCT03655678), using CRISPR/Cas9 to disrupt the erythroid-specific enhancer of the BCL11A gene, aiming to increase γ-globin levels and ameliorate the disease (Figure 2).

For several PIDs, gene editing using HDR may offer a safer therapy by avoiding aberrant gene expression from viral vectors, particularly useful for disorders where, for example, signaling molecules are affected and aberrant expression could be detrimental. Again, SCID was the first model in which proof of concept for this...
technology was shown. Genovese et al106 showed the feasibility of this approach; X-SCID HSC were corrected using ZFN and nonintegrating lentiviral vectors, which gave rise to functional lymphoid cells in an in vivo mouse model. In recent years, CRISPR/Cas9 and adeno-associated virus serotype 6 (AAV6) homology donors have risen to be the most promising tools, capable of correcting HSC to levels approaching 50% in vitro.107 Trials will determine the efficacy of these approaches in man.

Non-SCID immunodeficiencies

Following on from the successes in early trials of gene therapy for SCID, the approach was applied to more complex immune disorders where a survival advantage of gene-corrected cells may not have been so prominent.

Wiskott-Aldrich syndrome (WAS) is caused by an absence of WAS protein, a major regulator of the actin cytoskeleton in hematopoietic cells necessary for immune function and platelet production. The disease is associated with a spectrum of clinical presentations including immunodeficiency, thrombocytopenia, eczema, and increased risk of malignancy. Results for HSCT in WAS have improved over the years with the most recent report showing an OS of 90% regardless of donor source, if patients are treated in the first 5 years of life. For older patients, OS drops to 66%, and both acute and chronic GvHD is a significant risk (27%–17%, respectively).108 A clinical trial initiated in 2006 using γRV vectors showed clear clinical benefit and restoration of immune function.109 However, 7 out of the 9 patients that reconstituted immune function developed leukemia and integrations around LMO2/MDS1/EVI1 proto- oncogenic loci were later identified.110 To move forward, several centers chose a SIN LV vector that incorporated a 1.6 kb segment of the endogenous WAS promoter to drive WAS protein expression that had shown efficacy in preclinical models.110,111 A reduced intensity (busulfan/fludarabine) conditioning regimen was also standardized across centers.61,65,112 These trials are ongoing, but at the most recent published follow-up (up to 5.6 y post-procedure), collective data shows 90% survival and significant clinical improvement with sustained multi-lineage gene expression, correction of immune deficiency and eczema, and ability to stop immunoglobulin replacement therapy (NCT01515462/NCT01347242—1 death/15 treated patients).63,65 Post-procedure autoimmunity is seen in both HSCT and gene therapy cohorts.108,111 One major difference between the outcome of HSCT and gene therapy for WAS is the resolution of thrombocytopenia, which is superior following stem cell transplantation, although modest improvement in platelet count following gene therapy does prevent hemorrhagic events. The exact reason behind this is unclear and may relate to the number of gene-corrected cells infused114 but it is clear that gene-corrected platelets exhibit normal function.111

Chronic granulomatous disease (CGD) affects around 1:200000 births and arises due to defects in the subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex. Expressed in phagocytic cells, this complex generates reactive oxygen species (including superoxide anion radical, hydrogen peroxide, and hypochlorite) and activates neutrophil proteases that kill the engulfed bacteria or fungi in phagocytic vacuoles. In its absence, severe infections and chronic inflammation result. Again, transplant results have improved with a recent report of 712 patients showing an OS of 85.7%, with reduced OS for adult patients (76%). However, GvHD remains a significant risk responsible for a third of the fatalities in this cohort.116 CYBB (cytochrome B-245 beta chain) mutations encoding Gp91phox (glycosylated 91-kDa glycoprotein) cause the most common X-linked form (65% cases), which has been the target for all trials to date.115 Despite a lack of survival advantage of corrected cells in this disease, low numbers of oxidase-positive neutrophils or residual levels of NADPH oxidase expression can confer a significantly increase survival,118,119 making X-CGD an appealing candidate for treatment with gene therapy. An early γRV trial, initiated in the mid-1990s, recruited 5 adult patients and was performed without conditioning. Although there were no severe adverse events, gene marking in the periphery was very low and transient.120 Subsequent trials in multiple centers incorporated a myeloablative conditioning regimen that increased engraftment and restoration of immune function; however, this effect was also transient, with most of the 12 patients losing NADPH oxidase expression after 3 months,67,69,21-123 Three patients that did achieve significant gene marking in neutrophils in a trial using a spleen focus forming virus (SFFV)-based LTR γRV were found to have integration events around proto-oncogenic loci (PRDM16 and MDS1/EVI1) and later developed myelodysplasia.124 Further studies revealed that LTR promoter elements were being methylated, leading to gene silencing, while the enhancer elements (and therefore mutagenic influence) were unaffected. To improve safety, efficacy, and longevity, SIN LV vectors were developed that aimed to provide preferential expression in myeloid cells and detarget expression from HSC. Studies have investigated myeloid-specific promoters,124,125 a minimal CYBB promoter coupled with myeloid-specific enhancers,126 and a myeloid-specific promoter used in parallel with HSC-expressed microRNA binding sites.127 However, the most widely adopted vector was constructed by fusing cathepsin G and c-Fes proximal regulatory sequences, with the aim of driving maximal expression during terminal myeloid differentiation.124 Murine studies confirmed NADPH oxidase expression from the vector closely mimicked the endogenous expression profile and gene silencing through methylation was not observed. Two trials using this vector have recently reported early findings for the 9 patients enrolled; while 2 patients succumbed to disease-related comorbidities, 6 out of the 7 patients alive had stable copy number and 16%-46% oxidase-positive neutrophils, with no evidence of transgene silencing or untoward clonal dominance, up to 3 years post-procedure (NCT01855685/NCT02234934).128 A similar approach has been adapted to another form of CGD caused by mutations in the NCF1 gene leading to abnormal P47phox expression with proof of concept demonstrated in a murine model.129 Clinical trials of this LV are anticipated to start in the near future.

Leukocyte adhesion defect type 1 (LAD-1) is characterized by severe life-threatening recurrent bacterial infections due to impaired migration of neutrophils to sites of infection arising as a result of defective membrane expression of CD18 integrin subunit encoded by the ITGB2 gene. Treatment with HSCT is necessary, as mortality rates are between 60% and 75% in infancy for the most severely affected patients.130,131 However, disease severity tightly correlates with the level of CD18 expression, indicating that even a low level of correction could significantly reduce mortality. An early trial using a γRV in 2 patients that did not receive a conditioning regimen failed, with no gene marked cells detectable in the periphery after 2 months.127 Subsequent studies using LV vectors in both murine132 and canine133 models have paved the way for a recently opened trial across Europe and the United States, using a busulfan conditioning regimen for patients without access to an HLA-identical sibling donor (NCT03825783, NCT03812263).

Gene therapy for primary immune deficiencies: future perspectives

Patients suffering from immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome exhibit severe autoimmunity due to mutation in the forkhead box P3 (FOXP3) gene. This transcription factor is considered a master regulator for successful development and function of regulatory T cells (Treg), that are vital for maintaining immune tolerance to self-antigens.134 Studies have shown that effector T cells can be converted to Treg by ectopic expression of FOXP3,135 and these

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Gene Therapy for Primary Immunodeficiency
cells exhibit suppressor function in vitro, and in mouse models of GvHD, offering the potential of a T cell therapy. However, generating sufficient numbers of Tregs for this purpose will be challenging and the life span of the cells in vivo is unknown. Correction at the level of HSC would provide a longer-lasting therapy, however, studies investigating this approach have noted that constitutive FOXP3 expression in HSC (where it is not usually expressed) had adverse effects on T cell differentiation and hematopoiesis. A recent study aiming to abrogate this effect by replicating the endogenous expression profile by harnessing 3 regulatory elements, the FOXP3 promoter and the 3′UTR (untranslated region) to regulate transgene expression, has shown promising results in vivo. Another recent study using gene editing tools to place FOXP3 cDNA under control of its native promoter reported partial correction of FOXP3 expression and suppressive function restored to within the lower range of healthy control cells, indicating this methodology could provide an alternative approach for IPEX patients.

Deficiency of the T cell costimulatory molecule CD40 ligand (CD40L) gives rise to X-linked hyper-immunoglobulin M (hyper-IgM) syndrome (XHIGM). CD40L expression is upregulated after T cell activation and is essential for T cell: B cell interactions that induce immunoglobulin class switching and antibody affinity maturation in B cells. The resulting lack of humoral immunity leaves patients vulnerable to bacterial and opportunistic infections and increased risk of malignancy and autoimmunity. HSCT is used to treat patients, but OS after this procedure is suboptimal with latest figures suggesting a 3-year OS of 78%, indicating the need for a better therapy.

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clinical trials, it will become easier for physicians to understand which patients may benefit from the different treatment options available.

**Note added after acceptance**

Since writing this manuscript, a patient treated with Strimvelis for ADA-SCID has been diagnosed with T cell leukemia. Causality is under investigation. For more information: https://ir.orchard-tx.com/news-releases/news-release-details/orchard-statement-options-available.

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**Disclaimer**

The views expressed are those of the author(s) and not necessarily those of the National Health Service, the National Institute of Health Research or the Department of Health, or Action Medical Research.

**Disclosures**

The authors declare no competing interest.

**References**


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