Treating Immunodeficiency through HSC Gene Therapy

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Haematopoietic stem cell (HSC) gene therapy has been successfully employed as a therapeutic option to treat specific inherited immune deficiencies, including severe combined immune deficiencies (SCID) over the past two decades. Initial clinical trials using first-generation gamma-retroviral vectors to transfer corrective DNA demonstrated clinical benefit for patients, but were associated with leukemogenesis in a number of cases. Safer vectors have since been developed, affording comparable efficacy with an improved biosafety profile. These vectors are now in Phase I/II clinical trials for a number of immune disorders with more preclinical studies underway. Targeted gene editing allowing precise DNA correction via platforms such as ZFNs, TALENs and CRISPR/Cas9 may now offer promising strategies to improve the safety and efficacy of gene therapy in the future.

Gene Therapy: An Odyssey

Gene therapy involves the transfer of a gene of interest to a relevant cell type to cure a disease. In the context of primary immunodeficiency (PID), this means using a viral vector to deliver a specific transgene (corrective cDNA) to autologous haematopoietic stem and progenitor cells (HSCs), thereby allowing the development of a functional immune system. The concept was pioneered as a curative treatment option for patients with rare, devastating immune disorders, namely SCID, Wiskott–Aldrich syndrome (WAS), and chronic granulomatous disease, lacking suitable donor haematopoietic stem cell transplantation (HSCT), which remains the current definitive treatment of choice. Furthermore, for some disorders, particularly SCID, a profound growth and survival advantage of transplanting 'corrected' HSCs and lymphoid progenitors has meant that clinical efficacy might be achieved without preconditioning the patient.

Gene therapy has been most extensively investigated as a treatment strategy for four PIDs: X-linked severe combined immunodeficiency (X-SCID), adenosine deaminase-deficient SCID (ADA-SCID), WAS and chronic granulomatous disease (CGD), all of which present different challenges in terms of achieving optimal correction and clinical efficacy. This type of therapy first reached the clinical arena in the early 1990s, using gammaretroviral (γRV) vectors to transfer a functional copy of the defective gene to patients with these severe immune deficiencies. The γRV approach was successfully used in these first clinical trials but, with the exception of ADA-SCID, several patients developed leukaemia or myelodysplasia stemming from the gene transfer procedure [1–6]. This was directly related to the design of the vector, where strong viral promoter enhancer elements were able to affect the regulation of cancer-related genes such as *LMO2*, *CCND2*, and *MECOM* (*MDS/EVI1* complex locus) leading to clonal expansion of transformed cells, a process termed insertional mutagenesis [1,2,6].

Further work demonstrated that accumulation of genetic lesions, rather than a single event, led to the development of leukaemia in these patients. However, the recognition of this mechanism led to the design of self-inactivating γRV and lentiviral vectors (SIN-γRV and SIN-LV), which did not contain harmful viral **long terminal repeat (LTR)** (see Glossary) sequences and instead incorporated alternative mammalian or endogenous promoters to drive transgene expression. Several of these vectors are now in Phase I/II clinical trials for all the above PIDs, and while they demonstrate efficacy comparable with that of early γRV trials, no adverse events related to clonal expansion have been reported [7–10]. Nevertheless, these initial studies proved that gene therapy could offer significant clinical benefit and improve patient survival, even if the promise of this treatment was overshadowed by adverse events. The purpose of this review is to highlight the results and clinical impact of more recent Phase I/II trials in PIDs; further background is available in other reviews. We also discuss the results from preclinical studies that have been developing curative gene therapy for an expanding range of immune disorders, describing how the emergence of gene editing technologies brings forth the potential to change the face of gene therapy by improving both safety and efficacy through targeted gene correction. Figure 1 (Key Figure) depicts an overview of the process and the evolution of retroviral vectors investigated in current clinical trials.

Treating SCIDs

Adenosine deaminase is an essential enzyme in the purine metabolism pathway and its deficiency leads to the accumulation of toxic metabolites. Thus, ADA-SCID is a multisystem disorder affecting not only the immune system, so patients may also manifest skeletal, neurological, and pulmonary symptoms. However, it is the severe immune deficiency that

causes the most significant clinical disease, with infants presenting with life-threatening infections due to a lack of T, B, and natural killer (NK) lymphocytes. Delivery of enzyme replacement therapy (ERT) with PEG-ADA can ameliorate the immune phenotype and allow systemic detoxification before a definitive procedure, but ERT alone is not considered a wholly effective long-term therapy [11]. ADA-SCID was the first PID to be treated with gene therapy, initially using autologous T lymphocytes and then HSCs with γRV vectors [12–15]. Over 40 patients with ADA-SCID have subsequently been treated with conventional γRV vectors since 2000, with 100% survival and 75% disease-free survival (meaning that they have not recommenced ERT or progressed to HSCT). The results of γRV-based trials undertaken in Milan, London, and the USA (UCLA/NIH) have been published and show persistence of gene-corrected cells, good immune reconstitution, and, importantly, metabolic detoxification [16–18]. Most patients enrolled in these studies received cytoreductive chemotherapy before infusion of gene-modified HSCs (busulfan 4 mg/kg or melphalan 140 mg/m^2), with PEG-ADA withdrawn before treatment to promote any survival advantage afforded by gene-corrected cells. As mentioned above, no severe adverse events related to insertional mutagenesis have been reported in any of these patients despite equivalent vector design and integration profiles similar to those seen in other trials in which they did occur. Although the specific reason for this is unclear, it is likely to represent a disease-specific phenomenon.

More recently, a SIN-LV construct was developed that contained **codon-optimised** ADA cDNA under the control of the elongation factor 1α short (EFS) promoter. This vector proved efficacious in preclinical studies [19] and entered clinical trials in 2012 in London and Los Angeles. The treatment protocol involves the harvest of autologous **CD34**$^+$ cells from bone marrow (BM) or of **mobilised** peripheral blood stem cells (PBSCs). It is increasingly evident

that reinfusion of higher numbers of gene-corrected progenitor cells improves the outcome and harvest of mobilised PBSCs through **leukapheresis** and allows the collection of larger numbers of $CD34^+$ cells. It is also clear that the use of reduced-intensity **conditioning** secures engraftment of gene-modified HSCs [20] and in these studies the patients received a single dose of busulfan (4–5 mg/kg). Preliminary results from the first 20 patients treated are promising [10]. The age of patients treated ranged from 5 months to 6.5 years, with cell doses of up to 17×10^6/kg. Moreover, good transduction efficiency [**vector copy number (VCN)** 0.25–6.3 copies/cell] was demonstrated in the final product that was returned to the patients. With a follow-up time of up to 3 years, excellent immune and metabolic recovery is evident, with most patients remaining clinically well and off ERT. Results show improved T cell numbers (including naïve T cells) and normalisation of proliferative responses concomitant with humoral recovery. Children with longer follow-up times have been able to stop immunoglobulin replacement therapy and have demonstrated antigen-specific responses to vaccinations [20]. Of note, integration site analysis has shown a polyclonal pattern of vector insertion sites [20]. Thus, the safety and efficacy of HSC gene therapy for ADA-SCID has now been proved over several trials, allowing gene therapy to stand as an alternative treatment even for patients who have a matched unrelated donor available for transplantation, as this treatment is associated with less use of chemotherapy and no risk of graft-versus-host disease. Consequently, it is likely that ADA-SCID will become the first *ex vivo* gene therapy treatment to be commercially licensed.

X-SCID is an X chromosome-linked inherited condition caused by defects in the common cytokine receptor gamma chain [IL-2 receptor gene (*IL2RG*)], a subunit required by IL-2, 4, 7, 9, 15, and 21 receptors. Early in infancy, patients typically present with severe infections and deficiencies in T, B, and NK cells, ($T^-B^+NK^-$ immunophenotype). To date, over 30

children have been treated with either γRV- or LV-mediated gene therapy in Europe and the USA with encouraging results and long-term benefit [7,21]. Between 1999 and 2006, 20 children were treated in Paris and London trials and 18 are alive today with sustained immune reconstitution [21–23]. These patients received autologous $CD34^+$ HSC progenitors transduced *ex vivo* with a Moloney murine leukaemia virus (Mo-MLV)-derived, γRV-containing *IL2RG* transgene. Due to the lack of conditioning, there was very little, if any, engraftment of gene-corrected, true HSC progenitors. Despite this, immune reconstitution has been sustained with near-normal numbers of functional T cells. Interestingly, a comparative study with haploidentical HSCT revealed superior rates and levels of reconstitution associated with autologous gene therapy [24]. This is noteworthy, as enhanced immune reconstitution may be important in the patient's early protection against infections. However, due to transactivation of proto-oncogenes (mainly *LMO2* and *CCND2*), five of the treated patients developed T cell acute lymphoblastic leukaemia (T-ALL) as long as 6 years post-gene therapy [1,2]. Four patients remain in remission following successful chemotherapy and one patient died [1,2].

Following the genotoxicity associated with this trial, the field focussed on improving the safety profile of the procedure through refinements in vector design. Figure 1 shows the evolution of safer vector configurations with progression from LTR-driven γRV vectors to SIN-γRV vectors and subsequently SIN-LV vectors, which are currently in clinical use. In 2010, parallel clinical trials for X-SCID using a SIN-γRV vector with *IL2RG* expression driven by the mammalian EFS promoter began in Europe and the USA, but again these were unconditioned procedures [7]. Preliminary results have been published for nine treated boys, suggesting an efficacy comparable with the γRV vector but with a significantly 'improved' integration profile (fewer insertions recovered close to proto-oncogenes) [7].

Patients have been monitored for up to 3 years, with eight boys surviving (one patient died of disseminated viral infection before immune reconstitution occurred). Seven boys have functional T cell recovery with major clinical benefit. Low gene marking was seen in one patient who subsequently received a mismatched unrelated cord blood transplant 8 months post-gene therapy. Although the follow-up duration for this cohort is limited, these results are highly encouraging and so far no vector-related adverse events have been reported. Currently, new trials are planned or under way where preparative chemotherapy is being used to potentially enhance humoral recovery to further improve patient outcome [25].

Gene Therapy for Non-SCID Immunodeficiencies

Non-SCID PIDs present numerous challenges in the context of HSC gene therapy; these conditions tend to have wider variability in clinical features and less survival advantage for gene-corrected cells and more intense conditioning is therefore required to secure long-term engraftment.

WAS is an X-linked condition arising from mutations in the *WAS* gene and is characterised by **microthrombocytopaenia**, eczema, infections, and autoimmunity. Boys also have an increased risk of malignancy [26]. In a similar fashion to gene therapy for SCID, early trials involving γRV-mediated gene transfer have been superseded by the use of SIN-LV vectors, again in response to the development of haematological malignancies in treated patients [3,4]. Genomic integrations were present in proto-oncogenes identified in other γRV trials, including *LMO2*, *CCND2*, and *MDS1*/*EVI1*. Six patients went on to develop T-ALL between 16 months and 5 years post-therapy and AML was described in three patients (one primary and two secondary) [3,4].

A SIN-LV vector containing a 1.6-kb fragment of the WAS promoter has subsequently been developed and has been used in clinical trials in Milan, London, Paris, and Boston since 2010 with the preliminary results published recently [8,9]. All studies used a combination of busulfan and fludarabine preconditioning. Twenty-one patients in total have now been treated and preliminary results suggest stable engraftment of gene-modified cells and a substantial clinical benefit. Platelet counts, bleeding episodes, severe infections, autoimmunity, and eczema were improved in most patients [8,9]. The highest levels of gene marking were achieved in lymphocytes, reflecting a survival advantage particularly for mature cells. In general, platelet recovery has been variable, perhaps reflecting suboptimal expression of the WAS protein in this lineage. However, higher cell doses have been associated with improved engraftment of gene-marked cells as well as partial resolution of thrombocytopaenia. In addition, there has been no evidence of genotoxicity at this relatively early time point and the results available so far are extremely promising [8,9].

CGD is caused by mutations affecting several proteins that comprise the NADPH oxidase complex, reducing the ability of phagocytes (particularly neutrophils) to kill bacterial and fungal pathogens. The most common mutations are found in the *CYBB* gene, which encodes the gp91phox protein, a key electron-transporting component of the NADPH oxidase complex. This form of CGD is inherited in an X-linked manner. Gene therapy trials to treat X-linked CGD were initiated in the mid-1990s in the USA and utilised a γRV vector similar to that used in X-SCID trials. Five patients were enrolled and received no preconditioning before infusion of gene-corrected cells [27]. Although transient improvement in neutrophil function and clinical benefit was demonstrated, this was not sustained (but also not unexpected), as restoration of gp91phox protein expression does not confer a selective advantage. Subsequent trials undertaken at several centres worldwide have used non-

myeloablative conditioning to improve engraftment and promote long-term clinical benefit using the same vector design [5,6,28–31]. In several patients, neutrophil function improved temporarily with up to 25% gene-marked neutrophils in the periphery, but due to a lack of sustained HSC engraftment the presence of functional neutrophils did not persist [5,6]. Four treated patients derived significant clinical benefit from gene therapy with persistently higher levels of functional neutrophils present in the blood [5,6]. This effect was in fact supported by clonal expansions related to γRV-mediated transactivation of the oncogenes *MECOM* (*MDS1/EVI1* complex locus*)* and *PRDM1* (Blimp-1) [5,6]. Furthermore, over time neutrophil function deteriorated and gp91phox protein expression was lost as a result of transgene silencing through methylation of the viral LTR [5,6]. In Frankfurt, the two adult treated patients developed myelodysplasia and consequently died from complications [6]. Two children treated in Zurich also developed mutagenic clonal expansions but received successful HSCT, one before developing frank myelodysplasia [31].

In response to the high risk of genotoxicity in these studies, safer vectors have been designed for CGD, namely SIN-γRV [32] and SIN-LV [33,34]. These vectors incorporate myeloid-specific promoter elements allowing high levels of gp91phox expression in terminally differentiated neutrophils while detargeting expression in HSCs to potentially reduce the risk of insertional toxicity. The vector currently under evaluation in multicentre trials is a SIN-LV configuration with a chimeric promoter formed from the myeloid-specific Cathepsin G and c-Fes regulatory elements [34]. This promoter element allows preferential expression in myeloid cell and differentiated granulocytes [34].

Early-phase clinical trials using SIN-configuration vectors are under way in Europe and the USA (Table 1). The range of inherited monogenic conditions treatable by HSC gene therapy is rapidly expanding and, although beyond the scope of this review, it is noteworthy that

clinical trials of SIN-LV vectors are either under way or planned for several other tractable conditions, including RAG1 and Artemis-SCID.

Preclinical Development of Lentiviral Gene Therapy for Inherited Immune Disorders

Given the encouraging results and clinical benefit seen in patients outlined above, gene therapy strategies are being developed for numerous other monogenic PIDs, several of which are nearing Phase I clinical trials. V(D)J recombination defects account for almost one-third of SCID cases and lead to a $T^{-}B^{-}NK^{+}$ phenotype due to an inability to generate T cell and B cell receptors, severely limiting lymphocyte development and the immune repertoire [35]. Causes include mutations in the recombination activation genes (*RAG1* and *RAG2*) and the *Artemis* gene. These forms of SCID are an important target for clinical gene therapy, as the results following HSCT for these conditions can be poor. Artemis-SCID is a DNA repair defect and thus is associated with radiosensitivity and an increased risk of malignancy. This is highly relevant not only for HSCT but for the development of appropriate gene therapy protocols.

Correction of a murine model of Artemis-SCID has been achieved using a SIN-LV vector with transgene expression under the control of the internal phosphoglycerate kinase (PGK) promoter [36,37]. Stable immune recovery of both T and B cells, comparable with levels observed in wild-type animals, was demonstrated at low copy number (VCN ~1 copy/cell). The effect of copy number has been particularly important for *RAG1* gene therapy. RAG1-deficient mice receiving a SIN-LV vector displayed functional immune reconstitution but clinically relevant efficacy was achieved only with high VCN, suggesting that internal regulatory sequences are currently insufficient for this condition [38]. However, the requirement for such a high VCN does raise concerns around the increased risk of

insertional mutagenesis, which would be an important consideration in terms of clinical translation. Further work has shown that HSC transduction at lower VCN leads to partial T and B cell recovery with reduced thymic cellularity and this is associated with the production of autoreactive T cells similar to those seen in patients with hypomorphic RAG-SCID (**Omenn's syndrome**) [39]. A *RAG2*$^{-/-}$ mouse model has also been corrected using a γRV and SIN-LV vector with a codon-optimised transgene to induce RAG2 expression [40,41]. As opposed to weak cellular promoters where B cell reconstitution was impaired, this RAG2 expression, which was driven by a ubiquitous chromatin opening element (UCOE), was able to restore T and B cell development and function in the murine model [40]. This provides support for clinical development of this type of vector design.

The importance of promoter choice has been illustrated in a number of PID models where a fine balance exists between allowing sufficient levels of transgene expression to correct the phenotype and eliciting cell toxicity due to overexpression of a tightly regulated molecule. Another PID example is leukocyte adhesion deficiency type 1 (LAD-1), which arises due to mutations in the leukocyte integrin CD18 and is characterised by severe bacterial infections. Two patients were treated with a γRV construct in 1999 in the USA but no sustained benefit was reported [42]. SIN-LV vectors containing various promoters such as PGK and EFS, both ubiquitous and disease specific (CD18, CD11b), have been investigated in a canine model of the disease (CLAD) [43–45]. However, disease recovery was suboptimal using the human EFS and PGK promoters compared with the murine stem cell virus promoter (MSCV) or human CD11b or CD18 promoter [43–45]. Sustained disease amelioration has been described after long-term follow up of dogs receiving CD34^{+} progenitors transduced with a SIN-**foamy virus** (and MSCV promoter) with no vector-related adverse events [46]. Ongoing studies using a

SIN-LV incorporating a chimeric myeloid regulatory element (as for X-CGD) are currently evaluating functional correction of the disease in murine models.

Transgene overexpression may also be problematic, as seen in the case of gene therapy for X-linked agammaglobulinaemia (XLA), where mutations in the BTK protein, essential for B cell development, lead to the absence of circulating B cells and antibodies. For instance, overexpression of BTK in a murine model was shown to lead to erythromyeloid proliferation independently from vector integrations [47]. In the same study, neither EFS nor CD19 (B cell specific) promoters were capable of rescuing B cell development. SIN-LV vectors have since been designed that do allow efficient and lineage-specific BTK expression through the use of the immunoglobulin μ enhancer (Eμ) in conjunction with the *Btk* promoter or immunoglobulin β promoter (EμB29 and EμBtk) [48,49]. The use of gene- or lineage-specific promoters should therefore allow a physiologically regulated expression profile, which is preferable when addressing disorders involving proteins such as signalling or activation molecules. Timing of expression proved highly important in preclinical studies aimed at correcting CD40 ligand deficiency, also termed X-linked hyper-IgM syndrome, characterised by abnormal cell-mediated immunity. Deficiency correction has been achieved in early γRV murine studies showing that CD40L can be upregulated by $CD4^+$ T cells on activation, although constitutive low-level *CD40L* expression was shown to lead to the development of T cell lymphoproliferation in 12 of 19 mice independently of insertional mutagenesis [50]. Nevertheless, the *CD40L* endogenous promoter can support an expression profile that recapitulates, to some extent, physiological CD40L protein levels. Thus, these results are highly relevant for the application of these promoters in the clinic [51,52].

Several PIDs in which the defect is limited to the lymphoid compartment, as in CD40L deficiency, immunodysregulation, polyendocrinopathy, enteropathy X-linked syndrome

(IPEX), and HLH, also lend themselves to corrective strategies employing transfer of autologous gene-corrected HSCs or T cells. Proof-of-concept studies for either a HSC or a T cell approach have been published or are under way for certain forms of familial haemophagocytic lymphohistiocytosis (FHL) (perforin deficiency [53] and Munc 13-4 deficiency, X-linked lymphoproliferative disease [54], and IPEX) [55]. Autologous T cell gene therapy is an attractive strategy as it has an established safety profile with hundreds of patients treated to date for haematological malignancies in cancer immunotherapy trials with no reported transformational effect. In addition, early trials of gene therapy for ADA-SCID have utilised gene-corrected peripheral T cells and persistent gene marking in these patients and demonstrated the longevity of specific populations of T lymphocytes, suggesting that long-term correction of the T cell compartment is possible [53–55].

Targeted Gene Correction

The rapid development of gene editing technologies over the past decade has pushed to the forefront numerous platforms able to mediate targeted correction of a defective gene *in situ*. Such techniques can therefore allow expression of the corrected gene from native regulatory elements and eliminate the risks of insertional oncogenesis (assuming no off-target effects). An increasing range of editing techniques is evolving but the most commonly used are zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system. All rely on the same premise to modify the gene of interest: each combine specific DNA-recognition sequences (to allow precise genomic targeting) with an artificial nuclease capable of generating a double-stranded break (DSB) in the DNA. Following DSB, repair is mediated either by nonhomologous end joining (NHEJ), an error-prone mechanism, or

through homologous recombination (HR), which repairs DNA accurately in the presence of an appropriate donor sequence [56]. Presently, the relatively low efficiency of repair by HR in HSCs limits the clinical application of this approach. However, the investigation of various delivery methods for the editing tools, donor DNA, and specific culture conditions may improve HSC-mediated clinical applications over time.

In the context of HIV infection or cancer, deleterious mutations in specific genes via ZFNs and TALENs are being tested in humans in ongoing studies in the USA and Europe [57]. This work has involved modifying patient T cells and although this approach would be transferable to some PIDs, correction of patient HSCs remains the ultimate aim. Successful correction of human haematopoietic progenitor cells has already been reported, which represents an important step in realising the treatment of PID patients through genome editing [58]. To pursue improved treatments for X-SCID, Genovese *et al.* were able to target the integration of corrective cDNA into the *IL2RG* locus in patient $CD34^+$ cells and demonstrate multilineage differentiation, as well as long-term immune reconstitution when transferring gene-corrected HSCs into NSG mice [58]. Using CGD as an example, patient-derived induced pluripotent stem cells (iPSCs) have also been utilised as an *in vitro* model to demonstrate successful genetic correction by means of ZFNs [59], TALENS [60], and CRISPR/Cas9 [61]. Although the efficiency of genome editing is a limiting factor for progression of HSC-based therapy in the clinic, T cells appear to be more amenable to this technology, as has been shown through clinical studies in patients with HIV and cancer [57,62]. As discussed above, specific PIDs may be treated by a gene-corrected T cell approach and therefore gene editing could be used in this setting. Studies using TALENs and CRISPR/Cas systems to correct CD40L deficiency and XLP are also under way.

Despite current challenges associated with these technologies (Figure 2), some of which primarily relate to low efficiency of correction and off-target effects, it is highly probable that, in the coming years, gene editing techniques will progress to clinical translation to treat severe PIDs.

Concluding Remarks

Somatic gene therapy of inherited immunodeficiency has in many ways led the way in the development of technologies that can now be applied to the treatment of a range of inherited disorders. The numbers of patients that have been successfully treated is approaching levels where this strategy can be considered as a frontline approach, in addition to more conventional allogeneic procedures. Efforts are now under way in several areas to develop commercial models that will enable much wider dissemination and adoption in health-care systems. New technologies, including gene editing in particular, offer the exciting promise of much more precise genetic correction or targeted gene addition but will have to tackle important issues related to safety, efficacy, and scalability before being widely applicable (see Outstanding Questions and Figure 2). The future appears particularly bright for *ex vivo* gene therapy and the next decade will undoubtedly see similar approaches being applied to a much wider range of tractable haematological and metabolic diseases.

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Glossary

CD34: a surface antigen marker selectively expressed on haematopoietic stem cells.

Codon optimised: certain codons are preferentially used by different species without disrupting the amino acid sequence. Altering codon usage towards preferred or abundant codons can lead to improved protein expression levels.

Conditioning: therapy (usually chemotherapy) used to prepare a patient for stem cell transplantation or gene therapy. The purpose is to make space in the BM niche to allow engraftment of donor or gene-corrected cells (myeloablative conditioning) and suppress the immune system to prevent immune-mediated complications such as rejection.

Foamy virus: a type of retrovirus (*Spumavirus*) capable of integrating in non-dividing cells in a similar fashion to gammaretroviruses and lentiviruses but with a different integration profile.

Leukapheresis: the process whereby mobilised HSCs are separated from the blood and collected for processing in the laboratory. Using a cell-separator machine, blood is removed from the patient, HSCs are separated, and the blood is returned to the patient.

Long terminal repeat (LTR): sequences of DNA found in the 5' and 3' ends of proviral DNA containing regulatory regions required to insert into a host genome. They include enhancer and promoter elements and transcriptional signals.

Microthrombocytopaenia: low numbers of small platelets.

Mobilisation: the process of increasing the number of HSCs produced by the BM and promoting release into the peripheral circulation through the use of specific agents (GCSF and plerixafor).

Omenn's syndrome: a condition associated with certain forms of SCID (RAG, Artemis) caused by immune dysregulation and inflammation. It is characterised by skin rashes

(typically erythroderma), hepatosplenomegaly, lymphadenopathy, and diarrhoea. Eosinophilia and oligoclonal expansions of T cell clones can be seen.

Vector copy number (VCN): the number of copies of viral sequence detected per cell. This allows quantification of the level of gene marking.

Table 1. Open Phase I/II Clinical Trials of HSC Gene Therapy for PIDs

Disease	**Vector**	**Promoter**	**Conditioning**	**Stem Cell Source**	**Centre**	**Recruiting Since**	**No Patients**	**ClinicalTrials.gov Identifier**
X-SCID	SIN-γRV	EFS	None	BM	Boston, Cincinnati, Los Angeles, London, Paris	2010	11	NCT01410019 NCT01129544 NCT01175239
	SIN-LV	EFS	Busulfan 6 mg/kg	PBSCs	Memphis, NIH Clinical Center Bethesda[a]	2010	5	NCT01306019
	SIN-LV	EFS	Busulfan 6 mg/kg	BM	Memphis, Seattle	2012	0	NCT01512888
ADA-SCID	SIN-LV	EFS	Busulfan 5 mg/kg	BM/PBSCs	London	2011	14	NCT01380990
	SIN-LV	EFS	Busulfan 4 mg/kg	BM/PBSCs	Los Angeles, Bethesda	2013	16	NCT01852071 NCT02022696
WAS	SIN-LV	WAS	RIC busulfan/fludarabine	BM/PBSCs	Milan	2010	8	NCT01515462

	SIN-LV	WAS	RIC busulfan/fludarabine	BM/PBSCs	Boston, London, Paris	2011	13	NCT01410825 NCT01347242 NCT01347346
CGD	SIN-γRV	Myeloid specific	Busulfan	PBSCs	Frankfurt	2013		NCT01906541
	SIN-LV	Chimeric	MAC busulfan	PBSCs	London, Paris, Frankfurt, Zurich	2013	1	NCT01855685
	SIN-LV	Chimeric	MAC busulfan	BM	Los Angeles, Boston, Bethesda	2015	1	NCT02234934

[a]This trial is recruiting patients aged 2–30 years.

RIC, reduced intensity conditioning; MAC, myeloablative conditioning.

Figure 1. Key Figure. Haematopoietic Stem Cell (HSC) Gene Therapy. HSCs are harvested from a patient either through bone marrow (BM) harvest or leukapheresis of mobilised peripheral blood stem cells (PBSCs). $CD34^+$ cells are selected using magnetic separation columns and cultured with cytokines [usually IL-3, stem cell factor (SCF), thrombopoietin (TPO), and Flt-3 ligand] before transduction with a specific viral vector. The length of the transduction protocol varies according to the disease and the vector. After a period of culture, transduced $CD34^+$ cells are re-infused into the patient, who may have received cytoreductive or myeloablative chemotherapy to secure engraftment of gene-corrected haematopoietic progenitors in the BM niche. Again, the intensity of conditioning at this stage varies with each disease. Initial trials have utilised gammaretroviral (γRV) vectors with corrective cDNA expression under the control of viral promoters in the 5' and 3' long terminal repeats (LTRs). However, adverse events related to vector design have led to the development and utilisation of self-inactivating (SIN)-γRV vectors and, subsequently, SIN-lentiviral (SIN-LV) vectors in which deleterious LTRs have been mutated and appropriate transgene expression (driven by internal mammalian promoters) has been allowed to ensue. In later vector designs, the transgene cDNA has sometimes been codon optimised (CO cDNA) to further improve gene expression.

Figure 2. Potential Factors Leading to Improved Safety and Efficacy of Haematopoietic Stem Cell (HSC) Gene Therapy. Developments in three main areas have the potential to improve both the safety and the efficacy of HSC gene therapy. Greater accessibility to treatment will allow more patients to be treated, thereby generating substantial data that can be used to further understand the effect of variables such as cell dose and levels of engraftment on outcome. This, along with the incorporation of technological advances – for example, those

related to integration site analysis – will also help improve our understanding of factors affecting both safety and efficacy. In the long term, the rapid rise of gene editing platforms capable of precisely correcting gene defects will undoubtedly play a greater role in improving the safety and efficacy of gene therapy procedures.