Gene therapy for inborn error of immunity – current status and future perspectives

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Purpose of review
Development of hematopoietic stem cell (HSC) gene therapy (GT) for inborn errors of immunity (IEIs) continues to progress rapidly. Although more patients are being treated with HSC GT based on viral vector mediated gene addition, gene editing techniques provide a promising new approach, in which transgene expression remains under the control of endogenous regulatory elements.

Recent findings
Many gene therapy clinical trials are being conducted and evidence showing that HSC GT through viral vector mediated gene addition is a successful and safe curative treatment option for various IEIs is accumulating. Gene editing techniques for gene correction are, on the other hand, not in clinical use yet, despite rapid developments during the past decade. Current studies are focussing on improving rates of targeted integration, while preserving the primitive HSC population, which is essential for future clinical translation.

Summary
As HSC GT is becoming available for more diseases, novel developments should focus on improving availability while reducing costs of the treatment. Continued follow up of treated patients is essential for providing information about long-term safety and efficacy. Editing techniques have great potential but need to be improved further before the translation to clinical studies can happen.

Keywords
gene editing, gene therapy, inborn errors of immunity

INTRODUCTION

Inborn errors of immunity (IEI) comprise a group of over 450 rare inherited disorders of the immune system characterized by an underdeveloped and/or functionally compromised immune system [1], leading to increased susceptibility to infections, autoimmunity, inflammatory disease, allergies, bone marrow failure and/or malignancies. Disease is often severe with early treatment required to limit severe morbidity or prevent death. Allogeneic hematopoietic stem cell transplantation (HSCT) offers a curative treatment option but is associated with the risk of graft versus host disease (GvHD) and transplantation related mortality and is dependent on the availability of a human leukocyte antigen matched donor. Correction of hematopoietic stem cells (HSCs) results in restoration of all hematopoietic lineages and therefore autologous HSC gene therapy (GT) may be a curative approach for monogenetic haematopoietic disorders such as certain IEIs. Of note, not all IEIs are monogenetic disorders or underlying genetic causes may be unknown. In particular IEIs with known causative mutations in single genes are ideal candidates for an autologous HSC GT approach. An autologous approach avoids the risks associated with allogeneic transplantation and the need to find a donor but it is not without risk. Here we review the most recent developments in HSC GT for IEIs, discuss long-term follow up clinical data that is now available and highlight current challenges and future directions.

In general, HSC GT encompasses the following steps (Fig. 1): patient HSCs are harvested; either through apheresis of mobilized HSCs from

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KEY POINTS

- Viral vector mediated gene addition is widely used in clinical trials now, showing long-term safety and efficacy of the treatment.
- Future developments should focus on decreasing costs and increasing availability of hematopoietic stem cell (HSC) gene therapy for inborn errors of immunity (IEIs).
- Gene editing provides a promising new approach to treat IEIs keeping transgene expression under the control of endogenous regulatory elements.
- Before gene editing can be used in clinical trials editing rates will need to be improved while maintaining the engraftment and self-renewal potential of the edited primitive HSCs.

peripheral blood, or through direct aspiration from the bone marrow. $CD34^+$ cells are selected, cultured and genetically modified *ex vivo*. The patient then undergoes conditioning to improve engraftment before infusion of gene corrected HSCs. These modified HSCs retain their self-renewing potential and thus genetic correction is maintained after cell division and established in multiple haematopoietic lineages. Level of correction required to ameliorate disease manifestations varies and is highly dependent on the disease. Similarly, the level of conditioning is guided by individual diseases depending on the need for lymphoid or myeloid engraftment, or both. For example, low dose busulfan conditioning is used in the context of severe combined immune deficiency (SCID) diseases but myeloablative conditioning has been used in chronic granulomatous disease (CGD) and Wiskott Aldrich syndrome (WAS). Given the autologous nature of the procedure, serotherapy is not required.

Viral vector mediated gene addition and gene editing are two distinct techniques at different stages of development with specific advantages and disadvantages. Viral-mediated gene addition results in the semirandom integration of one or more copies of the therapeutic gene in the host

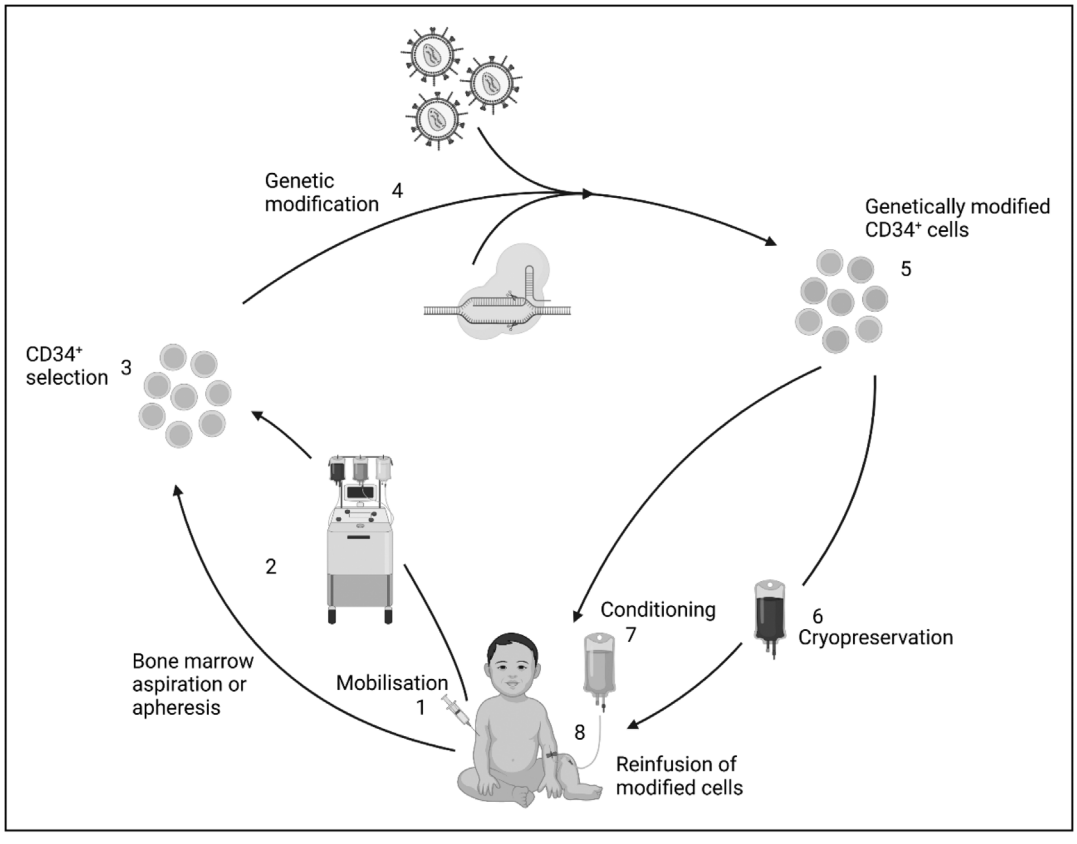

FIGURE 1. Hematopoietic stem cell (HSC) gene therapy (GT) [1]. In case of apheresis, the patient undergoes treatment with G-CSF and/or Plerixafor for stem cell mobilization [2]. HSCs are collected through bone marrow aspiration or apheresis of mobilized HSCs from the peripheral blood [3]. $CD34^+$ cells are selected and cultured *ex vivo* [4,5]. Selected $CD34^+$ cells are genetically modified through viral vector mediated gene addition or gene editing [6]. Genetically modified $CD34^+$ cells are sometimes cryopreserved [7]. The patient receives conditioning, the conditioning regimen used is dependent on the specific disease [8]. The genetically corrected autologous $CD34^+$ cells are reinfused into the patient. Created with BioRender.com.

DNA. Transcription is driven by a synthetic promotor that is incorporated in the vector. The first GT clinical trials were performed in the 1990s [2–4] and since then, great progress, improving both efficacy and safety, has been made. Today, over 300 IEI patients have successfully been treated with autologous GT in multiple clinical trials. Gene editing, on the other hand, is based on the creation of a targeted double strand break (DSB) in the DNA. With the introduction of a homology donor, the cell can be driven towards homology directed repair (HDR). By incorporating the functional copy of a gene in the donor template, targeted gene correction can be achieved. The transgene is *in frame* with the coding sequence and remains under the control of the endogenous promotor and other regulatory elements, which is advantageous for diseases where gene expression is tightly regulated. However, gene editing for IEIs is still in a preclinical phase and no knock-in strategy has reached the phase of clinical trial yet.

MAIN TEXT

Vector-based gene addition

Data from early GT clinical trials in certain forms of SCID and X-linked chronic granulomatous disease (X-CGD) using gamma retroviral (γRV) vectors revealed low efficiency and poor engraftment. Improvements in *ex vivo* culture and gene transfer techniques resulted in higher levels of correction [5], whereas the introduction of conditioning improved engraftment [6]. Subsequently, HSC GT was developed for more IEIs, but significant safety issues arose when patients developed leukaemia as a result of insertional mutagenesis [7–9]. This led to the development of safer gene-delivery platforms by: mutating long terminal repeat sequences creating self-inactivating (SIN) viral vectors [10–12], inserting a less powerful mammalian promotor driving transgene expression [13], use of lentiviral (LV) vectors with an integration pattern that has lower risk of oncogene-activating insertions compared to γRV vectors [12,14]. These changes have led to safer, more effective therapies and restored optimism in gene therapy. The licensing of Strimvelis in 2016, the first gene therapy product to be licensed for adenosine deaminase-SCID (ADA-SCID), marked a significant turning point in the field [15]. A more detailed timeline of the development of GT for IEIs is given by Ferrari *et al.* [16] and all currently active GT clinical trials for IEIs are listed in Table 1.

Severe combined immune deficiency

SCID is characterized by severe impairment in the development and function of T-lymphocytes combined with natural killer (NK) cells and/or B-lymphocyte deficiencies. At present, multiple clinical trials are ongoing or have been completed for various forms of SCID.

Adenosine deaminase deficient SCID

ADA-SCID is characterized by deficiency of T- and B-lymphocytes and NK cells due to the absence of adenosine deaminase and the subsequent accumulation of toxic metabolites. Without treatment ADA-SCID is fatal in infancy. ADA-SCID is the first IEI for which HSC GT is considered alongside allo-HSCT as first line treatment, when a matched donor is unavailable [27–29]. ADA-SCID was the first IEI for which GT was developed and initial trials using a γRV vector [2–4] show 100% survival up to 13.4 years posttreatment, with immune reconstitution in >80% of the patients [30,31▪]. Despite evidence of vector integration sites adjacent to proto-oncogenes and reports of leukemogenesis in patients with other IEIs [31▪,32,33], no γRV vector-related serious adverse events in ADA-SCID were described until 2018 when a case of leukaemia was reported 4.7 years posttreatment with Strimvelis [34]. Subsequent multicentre trials using a SIN-LV vector show excellent results in over 50 patients, with 100% survival, >95% persistent engraftment, and immune reconstitution with >90% of the patients discontinuing enzyme replacement therapy and immunoglobulin-replacement therapy (IgRT). No vector related adverse events or concerning monoclonal expansions were observed [17▪].

X-SCID

X-linked SCID (X-SCID) is caused by a defect in the *IL2RG* gene, which encodes for the common cytokine receptor γ chain [35], leading to the absence of T-lymphocytes and NK cells and defective B-lymphocytes. Early HSC GT trials for X-SCID using γRV and no preparative chemotherapy showed effective reconstitution of T-lymphocyte immunity in most patients [5,36,37], but some patients developed leukaemia as a result of vector driven insertional mutagenesis [7–9]. Safer SIN-γRV and SIN-LV vectors were developed [10,19] and busulfan conditioning was introduced to promote a more complete immune reconstitution, particularly humoral immunity allowing freedom from IgRT [19]. An overview of all GT trials for X-SCID is given by Pai and Thrasher [38]. Most recently, LV vector-based GT combined with low-dose busulfan conditioning showed persistent engraftment allowing for long-term reconstitution of functional T- and B-lymphocytes and NK cells resulting in clinical improvement [20▪].

Table 1. Active *ex vivo* gene therapy clinical trials for IEIs

Disease	Clinical trial registry number	Vector	Site	Cryopreservation	Promotor	Transgene	Conditioning	Reference
ADA-SCID	NCT03765632	SIN-LV *EFS-ADA*	London	Yes	EFS	Codon-optimized ADA	Low-dose Busulfan	
	NCT02999984	SIN-LV *EFS-ADA*	Los Angeles	Yes	EFS	Codon-optimized ADA	Low-dose Busulfan	[17▪]
	NCT04140539	SIN-LV *EFS-ADA*	Los Angeles	Yes	EFS	Codon-optimized ADA	Low-dose Busulfan	
	NCT03645460	SIN-LV *TYF-ADA*	Shenzhen		Unknown	ADA, not specified	Unknown	
	NCT05432310	LV *EFS-ADA*	Los Angeles	Yes	EFS	Codon-optimized ADA	Low-dose Busulfan	
X-SCID	NCT01129544	SIN-γRV *pSRS11.EFS.IL2RG.pre*$^{\times}$	Boston, Los Angeles, Cincinnati		EFS	IL2RG	None	[10]
	NCT01306019	SIN-LV *CL20-4i- EF1a- hyc-OPT*	Bethesda		EF1α	Codon-optimized IL2RG	Low-dose Busulfan	[18,19]
	NCT03315078	SIN-LV *CL20-4i- EF1a- hyc-OPT*	Bethesda		EF1α	Codon-optimized IL2RG	Low-dose Busulfan	
	NCT01512888	SIN-LV *CL20-4i- EF1a- hyc-OPT*	San Francisco, Memphis, Seattle	Yes	EF1α	Codon-optimized IL2RG	Low-dose Busulfan	[20▪]
	NCT03217617	SIN-LV *TYF-IL-2Rg*	Beijing, Shenzhen		Unknown	IL2RG, not specified	Unknown	
	NCT03601286	SIN-LV *G2SCID*	London	Yes	EFS	Codon-optimized IL2RG	Low-dose Busulfan	
	NCT03311503	SIN-LV *G2SCID*	Los Angeles, Atlanta, Boston, Cincinnati, London	Yes	EFS	Codon-optimized IL2RG	Low-dose Busulfan	
	NCT04286815	SIN-LV Unknown	Chongqing		Unknown	IL2RG, not specified	Unknown	
Artemis SCID	NCT03538899	SIN-LV *AProArt*	San Francisco	Yes	APro	DCLRE1C	Sub-ablative Busulfan	[21▪]
	NCT05071222	SIN-LV *G2ARTE*	Paris		Unknown	DCLRE1C	Busulfan	

Table 1 (*Continued*)

Disease	Clinical trial registry number	Vector	Site	Cryopreservation	Promotor	Transgene	Conditioning	Reference
RAG1-SCID	NCT04797260	SIN-LV *pCCL.MND.coRAG1.wpre*	Leiden	Yes	MND	Codon-optimized RAG1	Bu/Flu	
WAS	NCT01515462	SIN-LV *OTL-103*	Milan		1.6 kb WASp	WAS	Bu/Flu (reduced intensity)	[22]
	NCT03837483	SIN-LV *OTL-103*	Atlanta, Milan	Yes	1.6 kb WASp	WAS	Bu/Flu (reduced intensity)	
	NCT01410825	SIN-LV *w1.6_hWASP_WPRE*	Boston		1.6 kb WASp	WAS	Bu/Flu (myeloablative)	[23]
	NCT02333760	SIN-LV *w1.6_hWASP_WPRE*	Paris, London		1.6 kb WASp	WAS	Bu/Flu (myeloablative)	
CGD	NCT01855685	SIN-LV *G1XCGD/OTL-102*	London		CatG/Cfes	Codon-optimized CYBB	Busulfan (myeloablative)	[24▪]
	NCT02234934	SIN-LV *G1XCGD/OTL-102*	Los Angeles, Bethesda, Boston	Yes	CatG/Cfes	Codon-optimized CYBB	Busulfan (myeloablative)	[24▪]
	NCT02757911	SIN-LV *G1XCGD2*	Paris	Yes	CatG/Cfes	Codon-optimized CYBB	Unknown	
	NCT01906541	SIN- γRV *Unknown*	Frankfurt		Unknown	CYBB, unspecified	Unknown	
	NCT03645486	SIN-LV *TYF-CYBB and TYF-NCF1*	Shenzhen		Unknown	CYBB, unspecified	Unknown	
	NCT00778882	γRV *MT-gp91*	Seoul			CYBB	Bu/Flu	[25]
LAD-1	NCT03812263	SIN-LV *Chim- CD18- WPRE/RP-L201*	Los Angeles, Madrid, London	Yes	CatG/Cfes	ITGB2	Busulfan (myeloablative)	[26]

ADA-SCID, adenosine deaminase severe combined immunodeficiency; Bu/Flu, Busulfan and Fludarabine; CatG/Cfes, chimeric CathepsinG and cFes; CGD, chronic granulomatous disease; EF1α, elongation factor 1 alpha; EFS, EF1α Short; LAD-1, leukocyte adhesion defect type 1; LV, lentivirus; MND, myeloproliferative sarcoma virus enhancer; SIN, self-inactivating; WAS, Wiskott-Aldrich syndrome; WASp, WAS promotor; X-SCID, X-linked severe combined immunodeficiency; γRV, gammaretrovirus.

Artemis SCID

Artemis SCID is caused by a mutation in the *DCLRE1C* gene resulting in V(D)J recombination defects and a T-B-NK+ SCID with increased sensitivity to alkylating drugs and irradiation [39–41]. Allo-HSCT is particularly challenging in this patient population due to high rates of rejection and GvHD, incomplete immune reconstitution and increased conditioning induced toxicity. After promising preclinical study results [42,43], the first five patients have been treated with a SIN-LV containing the *DCLRE1C* transgene driven by the human endogenous Artemis promotor in a phase I/II clinical trial. All three evaluable patients showed multilineage gene marking and reconstitution of T-lymphocyte immunity [21▪].

RAG-1 SCID

Recombination-activating gene (RAG1) deficiency, like Artemis SCID, leads to impairment of V(D)J recombination and thus T- and B-lymphocyte dysfunction. *RAG1* expression is tightly regulated, so preclinical studies initially focussed on gene expression levels and various promotors driving *RAG1* expression [44–46]. Following positive preclinical results using a SIN-LV with a MND promotor driving transgene expression [47], the first RAG1-SCID patient has now been treated and at 9 months after gene therapy, the clinical course has been uneventful with stable T and B cell reconstitution (A. Lankester, personal communication).

Other forms of SCID

GT for other forms of SCID have not yet reached the clinic but early phase preclinical murine studies focussing on GT for IL7R-SCID [48] and RAG2-SCID [49] have been performed. Following a failed attempt of HSC GT in a JAK3 deficient patient [50] no recent studies have been published on gene therapy for this disease.

Non-SCID disorders

The development of HSC GT for IEIs is not limited to SCID variants. Clinical trials of GT for WAS, X-linked chronic granulomatous disease (X-CGD) and leukocyte adhesion deficiency type I (LAD-1) are currently active, while preclinical studies are focussing on various other diseases.

Wiskott-Aldrich syndrome

The WAS protein regulates polymerization of actin. WAS deficiency leads to recurrent infections, severe eczema, microthrombocytopenia, autoimmunity and an increased risk of developing lymphoid malignancies [51]. As in X-SCID patients, leukemogenesis was reported in WAS patients who were treated with γRV vector-based HSC GT [52,53]. Safer SIN-LV vectors were developed [22,54–56] and clinical follow up data show an overall survival rate of 91% and 89% up to 9 years posttreatment in two different studies [57,58▪]. In all surviving patients sustained multilineage engraftment was observed, which led to clinical improvement. In the majority of the patients, platelet counts remained subnormal but sufficient to prevent spontaneous bleeding events and obviate the need of platelet transfusions [23,57–59]. Latest long-term data recently presented shows a favourable profile with respect to postprocedure autoimmunity (as seen after HSCT) and no increased risk of malignancy to date, suggesting that 'partial' correction is sufficient to provide durable improvement in disease manifestations.

Chronic granulomatous disease

CGD is caused by mutations in the phagocyte NADPH oxidase complex, leading to impaired production of reactive oxygen species and clinical manifestations of severe recurrent infections, granulomatous inflammation and inflammatory bowel disease [60]. Of particular importance for successful GT for CGD is sufficient gene marking in the myeloid lineage. Initial attempts at HSC GT for CGD using γRV platform were unsuccessful due to poor engraftment [61], insertional mutagenesis leading to myelodysplastic syndrome with monosomy 7 [62,63], and silencing of the transgene [62,63]. Follow-up data from the first 9 X-linked CGD patients treated with a new, safer, LV vector containing a chimeric promoter to preferentially drive transgene expression at high levels in myeloid cells [64–67], following myeloablative conditioning, showed 78% survival, no CGD-related infections posttreatment, and 67% of the patients could discontinue antibiotic prophylaxis [24▪]. Adult patients deemed unsuitable for HSCT on the basis of poor clinical status and end organ damage have benefitted from GT in these trials, demonstrating the feasibility of this therapy in an older patient population in need of alternative treatments. Given our knowledge from X-CGD female carriers, full correction is not necessary to ameliorate disease and neutrophil function over ~20% confers protection from severe infection, although mild inflammatory symptoms can sometimes be seen in female carriers at this level.

LAD-1

Leukocyte adhesion deficiency type (LAD-1), caused by mutations in the *ITGB2* gene encoding for CD18, is a rare disorder of leukocyte adhesion and migration. It is characterized by severe, life-threatening

infections and impaired wound healing [68]. A LV vector containing a chimeric myeloid promotor has been developed [69,70] and the first seven patients have been treated with this vector after myeloablative busulfan conditioning. First results indicate durable neutrophil CD18 expression accompanied by clinical improvement, without any vector related adverse events [26].

Other IEIs

More other inborn errors of immunity (IEIs) are the focus of preclinical GT studies, including: immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome [71,72], X-linked agammaglobulinemia (XLA) [73], IFNgR1 deficiency [74], familial hemophagocytic lymphohistiocytosis (FHLH) including perforin deficiency and FHL type 3, [75–80], X-linked lymphoproliferative disease (XLP) [81], ataxia-telangiectasia [82], severe congenital neutropenia (SCN) [83,84], X-linked hyper-immunoglobulin M (IgM) [85], and XIAP deficiency (C. Booth, personal communication). Some of these diseases pose additional challenges for an HSC GT approach, including lineage specific gene expression (XLA), tight regulation of gene expression (X-linker hyper-IgM), large gene size (ataxia-telangiectasia), and nonimmunological manifestations that are not cured by HSC GT (ataxia-telangiectasia).

New developments and future perspectives

Improved transduction protocols are aiming to increase transduction efficiency and decrease the amount of virus required to transduce cells, thus reducing cost. Use of poloxamer F108 (LentiBoost) a transduction enhancer has been incorporated in clinical trials, while other reagents, such as cyclosporine H are studied in a preclinical setting [86]. While lentiviral vectors have not been associated with leukemogenesis in IEIs, a recent case of leukaemia in a patient with adrenoleukodystrophy who was treated with a SIN-LV was reported [18]. Thus, future, long-term follow up studies are crucial, to assess long term safety of viral vector mediated gene addition.

However, with numbers of treated patients and years of follow-up increasing, evidence showing the long-term efficacy and safety of HSC GT is accumulating. It is likely that HSC GT will get an increasingly more prominent place in treatment algorithms for IEIs and even become first-line treatment for certain diseases. Moreover, HSC GT has been shown to be effective and safe in adolescent and adult patients [17▪,24▪,87], who often suffer from disease-related comorbidities resulting in inferior outcome postallogeneic HSCT. That said, it must be noted that in general outcome of allo-HSCT is improving with new graft manipulation techniques and improved and personalized conditioning regimen. Thus, it is important that up-to-date data on outcome post-HSC GT and allo-HSCT is considered by clinicians when deciding upon a treatment of choice for their patients.

Now, logistical issues and cost benefit considerations are becoming increasingly relevant. The complexity of conventional LV gene therapy with *ex vivo* cell manipulation in specialized GMP facilities and the high cost of reagents required in the process, contributes to the extremely high-cost price of licensed therapies and research is underway to help reduce these costs. As the infrastructure of the manufacture of *ex vivo* GT products expands, for example with the development of automated good manufacturing practice-compliant methods and centralized manufacturing facilities [88,89], costs may drop and availability increase. The use of cryopreserved products, already widely used in clinical trials, shows no safety issues [20▪,21▪,24▪] and will further improve availability. Also, *in vivo* HSC GT approaches, which can be administered immediately after mobilization of HSCs and for which no conditioning is required, are being investigated in preclinical studies [90–93]. *In vivo* treatment might be particularly suitable for disorders in which the corrected cells have a survival advantage. Thus, we believe that HSC GT will not only become available for a broader range of IEIs, but that new developments will also improve availability to patients all over the world. Nonetheless the price of therapies that achieve marketing authorization will be prohibitive for many countries, reducing access to these therapies for patients in need.

Gene editing

Editing is based on the creation of targeted DSBs in the DNA and consequently forms a versatile technique with multiple applications. Upon creation of the DSB a cell has two main endogenous repair pathways: nonhomologous end joining (NHEJ) and homology director repair (HDR). NHEJ, the preferred pathway, is error prone and as a result small insertions and deletions are introduced and thus this is used to knock-out pathological dominantly active genetic elements. When a homology donor is introduced, the cell is directed towards HDR. By integrating the entire corrective cDNA in the donor cassette, HDR-mediated site-specific gene insertion leads to functional correction of disease-causing mutations throughout the gene. Targeted gene editing has the advantage of gene expression remaining under the control of the endogenous

promotor and other regulatory elements, preserving physiological expression which is essential in certain diseases. Finally, site-specific correction of disease-causing mutations, by introducing a single-strand oligodeoxynucleotide (ssODN), is useful if a single or predominant mutation causes disease.

Three main editing platforms have been developed. Zinc finger nucleases (ZFNs) [94,95] and transcription activator-like effector (TALE) nucleases (TALENs) [96,97] consist of a DNA-binding protein, zinc fingers or TALE modules respectively, with a defined recognition sequence that is fused to a nonspecific nuclease domain. Therefore, Zinc fingers and TALENS rely on protein-DNA interaction for site specific binding. In contrast, short guide RNA sequences are responsible for guiding the Cas endonuclease to the target site. Therefore, application of the CRISPR/Cas system [98] is easier, as target site recognition is based on Watson-Crick base pairing, while the Cas nuclease is identical for each targeted site and is now widely commercially available and generally the preferred platform for preclinical studies.

In proof-of-principle studies, it has been shown that HSC editing techniques can fully recapitulate a physiological gene expression pattern [99,100▪] and restore a functional immune response [101▪]. Furthermore, patient-derived edited HSCs can engraft and differentiate into various immune cells *in vivo* [101▪,102▪]. However, despite the rapid developments made in gene editing, clinical translation is slow and significant challenges remain including efficiency. We will discuss the most important recent developments that have been made in editing for IEIs and highlight the current problems that still need to be tackled.

Wiskott-Aldrich syndrome

After two proof-of-principle studies showed that targeted gene insertion at the *WAS* locus is feasible, although with low efficiency, and results in physiological WASp expression levels in edited patient derived iPSCs [103,104], a recent study using the CRISPR/Cas platform and an adeno-associated virus type 6 (AAV6) donor cassette demonstrated much higher rates of targeted integration, reaching approximately 60% [101▪]. WASp expression was restored to physiological levels, which resulted in restoration of functional defects in myeloid and lymphoid cells *in vitro* and *in vivo*. Long-term engraftment and preservation of the differentiation potential of edited HSPCs was shown *in vivo*. Furthermore, normal levels of WASp were observed in successfully edited megakaryocyte progenitors and mature platelets and *in vitro* studies suggested the correction of platelet-intrinsic defects [101▪].

X-linked hyperIgM syndrome

Editing is particularly suitable for X-linker hyperIgM syndrome, as CD40L expression is tightly regulated with upregulation on the surface of activated T-lymphocytes. Both a T-lymphocyte and an HSC approach have been studied, with the former being easier to translate and potentially useful as a bridging therapy [105▪]. Both strategies have been successful, resulting in restored physiologically regulated CD40L expression and functional correction *in vitro* [99,105▪]. Targeted gene correction in HSCs showed up to 25% editing efficiency, physiological CD40L expression levels and successful engraftment of edited HSCs in a mouse model [105▪,106].

X-SCID

Low editing efficiencies were observed in early attempts at gene editing for X-severe combined immune deficiency (X-SCID) [107]. Improvements in delivery methods and optimization of culturing conditions and editing timing increased editing rates to 20% in human HSCs [108,109]. Recently, editing rates of up to 47% were achieved in human HSCs using ZFNs or CRISPR/Cas nucleases and AAV6 donor cassettes in optimized protocols [102▪,110]. Multilineage reconstitution was shown *in vitro* and *in vivo* and off-target indels were below the limit of detection [102▪]. Because of the survival advantage edited cells have, relatively low editing rates will be sufficient for treatment of X-SCID [110].

Chronic granulomatous disease

Various editing approaches have been attempted for CGD, including: targeted insertion of the *CYBB* transgene at a safe harbour using donor cassettes containing various promotors [111,112], site-specific repair of a missense mutation in exon 7 of the *CYBB* gene using CRISPR and a ssODN donor template [113], and targeted gene-correction of the most common two-nucleotide deletion causing p47-CGD using ZFNs [114]. All these approaches resulted in correction of cells, albeit at various degrees of efficiency, and in reconstitution of oxidase activity in corrected cells. An optimized editing approach for CGD using the CRISPR/Cas system showed that intron 1 is essential for restoration of physiologic $gp91^{phox}$ levels and that transient inhibition of NHEJ using i53 mRNA increases targeted correction [100▪].

Other IEIs

With experience growing, more IEIs have become subject of gene editing studies, including XLA [115], X-linked lymphoproliferative disease (XLP) [116], XMEN disease [117], RAG2-SCID [118], and SCN [119].

New developments and future perspectives

Optimizations and improvements of editing strategies now focus on improving editing rates while preserving the primitive HSC population to balance curative levels of protein expression with maintaining engraftment and long-term repopulating potential of edited HSCs.

Delivery methods of the nuclease and the homology donor are of importance. ZFNs and TALENs must be delivered as plasmid DNA or mRNA. CRISPR can be delivered as RNP, which results in higher editing rates [120]. Nonintegrating viral vectors, such as AAV and integrase-deficient lentiviral vector (IDLV), are generally used for delivery of the homology donor. Where IDLVs have a superior packaging capacity, they also have a nonnegligible rate of semi-random integration and low-titres [121]. Alternative, less toxic delivery methods are being investigated, including gold nanoparticles and virus like particles, but often at the cost of efficiency [122]. Strategies to improve efficiency under investigation include small molecules to inhibit NHEJ [123] or enhance HDR [124], or upregulation of components of the HDR machinery [125▪].

Editing of true, more primitive, quiescent HSC populations is relatively inefficient and their ability to engraft and self-renew is impaired after manipulation. As HDR occurs mainly during the S/G2 phase of the cell cycle, more quiescent HSCs are more likely to undergo NHEJ. Transient p53 inhibition and forcing cell-cycle progression improves editing efficiency [125▪]. Furthermore, HSCs are likely to be sensitive to DSBs, impairing their ability to engraft and self-renew. The use of small molecules, such as UM171, PGE2 and StemRegenin1 are used to expand HSCs in culture and promote self-renewal potential [102▪,108,120,126].

Donor design influences results and for some genes intronic elements have been shown essential for optimal expression [100▪]. Various regulatory sequences, such as the 5′ and 3′ UTR, Kozak sequences, transcription factor binding sites and Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), can be integrated into the donor cassette to improve expression.

Safety of the treatment is paramount and must be ascertained thus genome wide detection of off-target activity is essential. *In silico* analyses form a first step, followed by unbiased genome-wide detection techniques, including CIRCLE-seq and GUIDE-seq. However, more sensitive and specific tools are needed. In addition, continuous improvements are being made on the editing tools, such as high-fidelity Cas9 variants, new Cas12a variants and chemical modifications of the gRNAs, to improve specificity. The clinical relevance of findings of *in vitro* genotoxicity studies will be borne out in early phase trials.

Gene editing is a highly personalized treatment option for rare disorders and will be costly. Therefore, once the first clinical trials show long-term efficacy and safety, financial considerations will become increasingly more relevant. It is likely that the cost of gene edited therapies will be similar to costs of conventional LV gene therapy limiting availability and undoubtedly more research will be undertaken to achieve high quality products at lower cost.

Concluding remarks

As the rapid developments continue, HSC GT will likely become a more common therapeutic option for IEIs. Efforts should be made to make viral vector mediated gene addition more readily available by improving infrastructure and reducing costs. Editing techniques have great potential, but still need to be improved in preclinical studies with successful scale up prior to the translation to clinical studies.

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Conflicts of interest

There are no conflicts of interest.

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