Gene Therapy for Primary Immunodeficiencies

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Abstract

Primary immunodeficiencies (PIDs) are a group of rare inherited disorders of the immune system. Many PIDs are devastating and require a definitive therapy to prevent progressive morbidity and premature mortality. Allogeneic haematopoietic stem cell transplantation (alloHSCT) is curative for many PIDs, and whilst advances have resulted in improved outcomes, the procedure still carries a risk of mortality and morbidity from graft failure or graft-versus-host disease (GvHD). Autologous haematopoietic stem cell gene therapy (HSC GT) has the potential to correct genetic defects across haematopoietic lineages without the complications of an allogeneic approach. HSC GT for PID has been in development for the last two decades and the first licensed HSC GT product for adenosine deaminase deficient severe combined immunodeficiency (ADA-SCID) is now available. New gene editing technologies have the potential to circumvent some of the problems associated with viral gene-addition. HSC GT for PID shows great promise but requires a unique approach for each disease and carries risks, notably insertional mutagenesis from gammaretroviral gene addition approaches and possible off-target toxicities from gene editing techniques. In this review, we discuss the development of HSC GT for PID, outline the current state of clinical development before discussing future developments in the field.

Introduction

Primary immunodeficiencies (PIDs) (also known as inborn errors of immunity) are a heterogeneous group of rare (1:10,000 births) inherited disorders of the innate or adaptive immune system (1, 2). More than 400 PIDs have been described and they are broadly classified into the following groups: combined immunodeficiencies, immune regulatory disorders, phagocytic defects, autoinflammatory disorders, defects in intrinsic or innate immunity, antibody deficiencies and complement deficiencies (3). Whilst a majority of patients present in childhood and adolescence, increasingly patients are being diagnosed later in life as an initial mild phenotype may not prompt investigation for PID (4).

Clinically PIDs have heterogeneous clinical presentations. Whilst recurrent infections (including severe and life-threatening infections) are the most common presenting complaint, an underlying PID should also be suspected in patients presenting with haematological and immunological symptoms at an earlier than expected age or which are refractory to conventional treatments. Haematological malignancies (particularly lymphoid malignancies), cytopenias, autoimmune phenomena such as idiopathic thrombocytopenic purpura or serious autoinflammatory complications such as haemophagocytic lymphohistiocytosis (HLH) can all be the presentation of a PID (5). Clinical severity varies widely from the desperately sick infant with severe-combined immunodeficiency (SCID) who requires urgent alloHSCT as a life-saving procedure, to the adult patient with common variable immunodeficiency (CVID) who has a normal life expectancy on immunoglobulin replacement therapy.

Whilst PID patients with a mild phenotype may maintain a normal life expectancy with supportive care alone, PIDs which result in life-threatening complications such as severe infections, malignancy or HLH, require a definitive therapy to prevent progressive morbidity and premature death. For SCID, alloHSCT is necessary for survival beyond infancy. For some of the commonest PIDs which are known to have an aggressive clinical course such as Wiskott Aldrich Syndrome (WAS) and chronic granulomatous disease (CGD), alloHSCT has become standard of care after diagnosis in many institutions. For less common PIDs that have a more variable clinical phenotype, decision to transplant is made on an individualised basis, usually prompted by the development of life-threatening complications.

Since the first alloHSCT for SCID was performed over 50 years ago, the field has progressed substantially and survival rates of over 95% have been achieved in SCID cohorts (6). The aim of transplant in a non-malignant setting differs, in that the patients do not stand to benefit from the graft-versus malignancy effect (apart from cases where they have developed a malignancy in the context of an underlying PID) and thus successful long-term engraftment with resolution of the clinical phenotype, whilst minimising the risk of GvHD is essential. Historically, a significant limitation on the availability of alloHSCT was a lack of a HLA-compatible donor with ~25% of patients having a HLA-identical sibling and less than 70% of others having a HLA-compatible unrelated donor (7). A major advance in the field has been the development of haploidentical alloHSCT protocols which permit the use of a donor that differs from the recipient by an entire haplotype, using T cell depletion techniques to prevent GvHD (8). The development of improved protocols such as posttransplant cyclophosphamide (PTCy) or graft manipulation with α/β T-cell and B-cell depletion have resulted in excellent overall survival with low rates of GVHD in nonmalignant cohorts that included patients with

PID (9-11). Early transplantation in PID is associated with better outcomes however an initial mild phenotype, late presentation, donor availability or other complications mean that a significant number of patients survive to adolescence and adulthood without a transplant. Historically older patients with PID had abysmal transplant outcomes but more recently, much improved survival rates for alloHSCT have been achieved in older patients with PID using reduced-intensity conditioning (RIC) (12, 13). There has been a paradigm shift in practice in recent years and patients who develop severe complications of PID later in life are increasingly considered for definitive treatment should their clinical scenario permit it (4).

Although, outcome following alloHSCT using alternative donors has improved, allogeneic procedures still carry a significant risk of the potentially catastrophic complications of graft-versus-host disease (GvHD), graft failure and graft rejection. Despite, improvements in overall survival rates, alloHSCT still carries a risk of TRM, particularly in older patients and in certain PIDs (5, 14, 15). For older patients with significant end-organ damage resulting from complications of their PID, the need for prolonged immunosuppression with an allogeneic procedure can preclude transplant as a viable treatment option (16).

The lack of immunogenicity associated with an autologous haematopoietic stem cell gene therapy (HSC GT) product abrogates the need for immune suppression as GvHD prophylaxis and removes the need to find a suitably matched donor (17). The discovery that retroviruses could be used as gene-carrying vectors, capable of integrating their payload into targeted cells resulting in protein expression, paved the way for development of HSC GT (18). PIDs were identified as the ideal candidates for the development of HSC GT due to the clear link between defined monogenic defects and a clinical phenotype of immune dysfunction, the ability to repair the defect in the immune cells by manipulating the readily accessible haematopoietic stem cell (HSC), and the survival advantage conferred (in many cases) to the genetically corrected cells.

HSC GT involves harvesting haematopoietic stem and progenitor cells that express the cell surface marker CD34, either by purification of bone marrow harvests (acquired by direct aspiration) or from apheresis of mobilised stem cells from peripheral blood (figure 1). Mobilisation of stem cells into the peripheral blood is most commonly performed using a combination of granulocyte-colony stimulating factor (G-CSF) and plerixafor (a reversible inhibitor of the binding of stromal cell derived factor-1 to its cognate receptor CXCR4) although disease specific limitations may be present, for example plerixafor alone is used for mobilisation in patients with sickle cell disease (19-21). HSCs are then harvested from the circulation through leukapheresis. Harvested CD34+ cells are selected and cultured *ex vivo* under conditions that favour long-term repopulating potential and gene transfer or gene editing performed under sterile conditions to correct the causative genetic defect (see figure 1) (22-24). As the cell divides, the genetic correction is maintained in the resulting progeny as they differentiate into haematopoietic lineages (25). The patient undergoes conditioning and the genetically modified HSCs are infused into the patient. The genetically modified HSCs hone to the bone marrow where they maintain their self-renewing potential and, as their progeny differentiate, the genetic modification or correction is conferred to all immune-haematopoietic lineages (26).

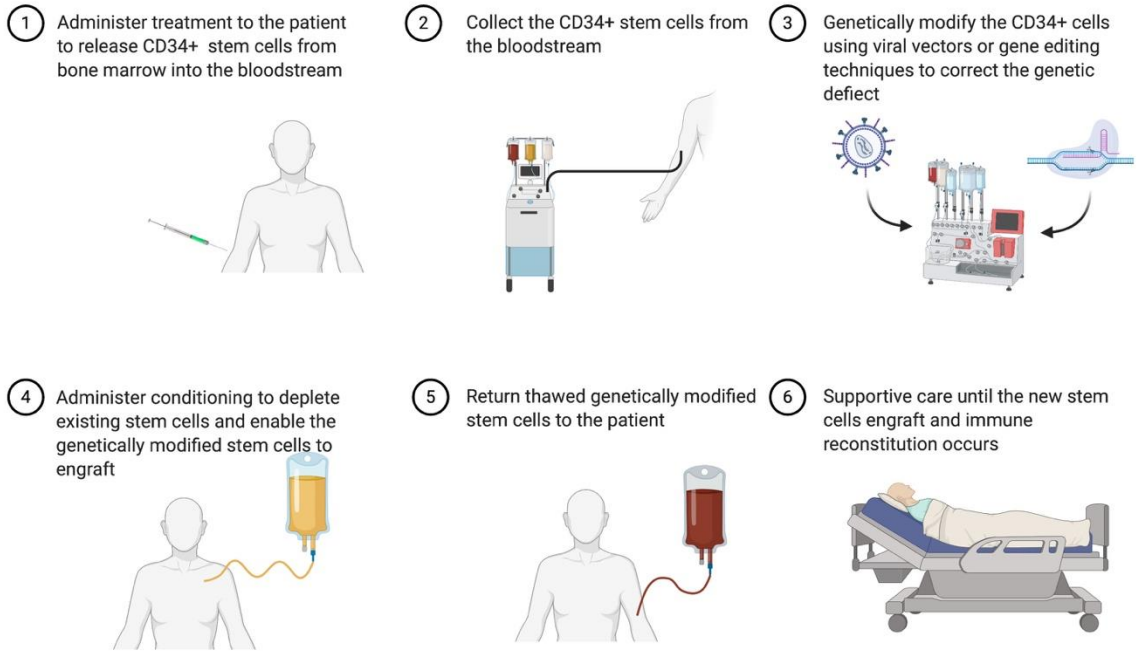


Figure 1: Schematic representation of the patient journey for HSC GT. (1) The patient undergoes stem cell mobilisation using G-CSF +/- plerixafor. (2) The patient undergoes apheresis to harvest HSCs from the peripheral blood. Direct aspiration from the bone marrow is an alternative to steps 1 and 2. (3) CD34+ stem cells are selected and cultured *ex vivo* in a sterile environment and genetically modified by viral gene addition or gene editing. (4) The patient undergoes conditioning with myeloablative or cytoreductive chemotherapy. Conditioning regimens vary between different PIDs and clinical trials. (5) The genetically modified stem cells are infused back to the patient where they hone to the bone marrow and engraft. (6) Conditioning regimens typically result in a period of severe cytopenias during which the patient requires supportive care/management of infections until engraftment of the modified stem cells occurs and immune reconstitution takes place. Created with BioRender.com

HSC GT for PID is finally 'coming of age' as increasing numbers of clinical trials report safety and efficacy data and the infrastructure to manufacture and deliver cellular therapies advances. Exciting developments such as gene editing technologies have the potential to improve the specificity of genetic correction and broaden the application for GT to genetically more complex PIDs. At the time of writing, 29 phase I/II HSC GT clinical trials are completed, in follow-up or are recruiting for common PIDs (SCID, WAS and CGD) (5). Molecular analysis clearly forms an important component in the evaluation of GT approaches and in trials of SIN-γRV and SIN-LV vectors, multilineage and polyclonal engraftment have been consistently demonstrated (27). Such significant progress has resulted in the first drug product receiving marketing approval in 2016. *Strimvelis®*, a SIN-γRV-based product is now available in Europe for patients with ADA-SCID who lack a suitable donor for alloHSCT (28). Several other products are in the advanced stages of clinical development and commercialisation.

Although HSC GT has many potential advantages over alloHSCT it also confers its own unique set of problems, notably the need for a bespoke approach for each disorder and the risks of

insertional mutagenesis seen with gammaretroviral gene addition strategies or potential off-target toxicities with gene editing techniques. These issues and the development of strategies to resolve them will be discussed in this review. As HSC GT approaches become available, the benefits and risks of these novel approaches in addition to financial and logistical considerations will need to be carefully assessed against contemporary alloHSCT outcomes to determine their place in treatment algorithms for patients with PID. In this review we explore the development, current application and future perspective of HSC GT for the different PID subgroups. Finally, we outline the latest developments in the field with a focus on gene editing strategies and discuss how these developments might change how we treat patients with PID in the future.

PID Classification	Disease	Phenotype	Genetic Defect/Inheritance	Treatment
Severe Combined Immunodeficiencies	Adenosine deaminase (ADA) deficiency	Presents in. infancy T- B- low NK cells, low Ig	*ADA* Autosomal recessive	AlloHSCT in infancy required (29, 30). SIN-RV based gene therapy available (*strimvelis*). SIN-LV HSC GT phase I trial reported (31).
	X-SCID	Presents in. infancy T- B+ low NK cells, low Ig	*IL2RG* X-linked	AlloHSCT in infancy required (5, 32). SIN-LV phase I clinical trial reported. HSC GT not yet licensed. Proof of concept gene editing approach (33, 34).
	Artemis deficiency	Presents in. infancy T- B- normal NK cell number, Low Ig, radiation sensitivity	*DCLRE1C* Autosomal recessive	Variable results to alloHSCT due to radiation sensitivity (35). Phase I clinical trial of SIN-LV HSC GT (NCT03538899).
	RAG Deficiency	Presents in infancy T- B- normal NK cell number, Low Ig	*RAG1* *RAG2* Autosomal recessive	AlloHSCT curative but increased risk of graft rejection due to activated NK cells (35). Phase I clinical trial of SIN-LV HSC GT in RAG1 deficiency expected to open soon (36).
Combined Immunodeficiencies	Wiskott-Aldrich Syndrome	Presents in childhood. Progressive decrease in T cell numbers, normal B cells, Low IgM, microthrombocytopenia	*WAS* X-linked	AlloHSCT curative. Outcomes inferior if aged >5(37, 38) . SIN-LV phase I clinical trial reported successful results although variable platelet correction (39). Proof-of-principle gene editing approach (40).
Disorders of Phagocyte Number and Function	X-linked Chronic Granulomatous Disease	Defective neutrophils and monocytes, results in recurrent infections and autoinflammatory phenotype. Most commonly presents in childhood but can present later	*CYBB* X-linked	AlloHSCT curative, increasingly performed once diagnosis established (14). Phase I clinical trial of SIN-LV HSC GT has been reported and clinical efficacy demonstrated (41). Gene editing strategy demonstrated in pre-clinical study (34).
	AR Chronic Granulomatous Disease	Similar to X-CGD	*CYBA, CYBC1, NCF1, NCF2, NCF4* Autosomal recessive	AlloHSCT curative (14). SIN-LV HSC GT approach for CGD caused by *NCF1* mutations in clinical development (42, 43).
	Leukocyte adhesion deficiency type 1	Defective neutrophils, monocytes, lymphocytes and NK cells	*ITGB2* Autosomal recessive	AlloHSCT curative (44). Phase I trials of SIN-LV HSC GT open in Spain and USA (NCT03825783, NCT03812263) (45).
Diseases of Immune Dysregulation	Familial haemophagocytic lymphohistiocytosis	Can present at any age (childhood more common). Increased activated T cells, decreased to absent NK cells	*PRF1, UNC13D, STX11, STXBP2, FAAP24, SLC7A7* Autosomal recessive	AlloSCT curative although requires control of HLH prior to transplant (46). Proof-of-principle SIN-LV approach published. Not yet entered clinical trials (47).
	IPEX syndrome	Lack of/impaired function of T regulatory cells	*FOXP3* X-linked	AlloHSCT curative (48). Proof of principle SIN-LV approach published. Not yet in clinical trials (49). Gene-editing pre-clinical work successful (50).
	X-linked lymphoproliferative disease 1	Normal or increased activated T cells, reduced memory B cells, reduced NK cell activity.	*SH2D1A* X-linked	AlloHSCT curative (51) Pre-clinical work with SIN-LV approach in T cells results in correction. Clinical trial planned (52).

Table 1: Summary of the presentation, genetic defect and treatment options including HSC GT for the most common PIDs.

Severe Combined Immunodeficiencies (SCIDs)

ADA-SCID

The ability to use viral vectors to insert a functional copy of the defective gene directly into the cellular chromosomal DNA was met with initial excitement. Adenosine deaminase deficient severe combined immunodeficiency (ADA-SCID), a devastating disorder resulting from the absence of an essential enzyme, adenosine deaminase was the first PID for which GT was attempted. This disease results in profound immunodeficiency with absence of T, B and NK cells and as a result, is fatal in infancy without alloHSCT. In 1990 the first clinical trial of HSC GT demonstrated that T-lymphocytes could be transduced with gammaretroviral (γRV) vectors containing the adenosine deaminase (ADA) enzyme. After infusion to a patient with ADA-SCID, production of the endogenous ADA enzyme occurred, resulting in amelioration of the clinical phenotype (53, 54). The use of modified T-lymphocytes required multiple infusions of the genetically modified product to maintain the therapeutic effects, a problem overcome by using gammaretroviral (γRV) vectors to introduce the ADA gene into HSCs which then engraft and self-renew with remarkable results (54-56). Metabolic correction and restoration of cellular and humoral immunity were demonstrated in these early trials (although PEG-ADA treatment at lower amounts continued throughout the study period) which led to great optimism (54). Ubiquitous expression of ADA makes it safe to use strong viral promoters in the γRV long terminal repeats (LTRs) to drive transcription of the gene, whilst the relatively small size of the *ADA* cDNA (1.5kb), simplified its incorporation into a viral vector (31).

Early trials using γRV vectors were performed without conditioning and the level of engraftment was insufficient for therapeutic ADA expression (57). Improved multi-lineage engraftment was observed with busulfan reduced intensity conditioning. The first pilot study of two ADA-SCID patients who lacked a HLA-matched donor and could not access enzyme replacement therapy, demonstrated that HSC GT could result in sustained engraftment of gene corrected cells with therapeutic ADA expression (29). In the later trials of the γRV vector developed in Milan, the majority (15/18) of patients remained off enzyme replacement and the oldest patient is now over 18 years old (58). This γRV product was approved for licensure by the European Medicines Agency under the name , *Strimvelis®*, providing an alternative therapy for patients who lack a suitable donor for alloHSCT (28). Whilst the approval of *Strimvelis®*, as the first HSC-GT product to receive licensing approval was an important milestone for the field, the use of the product is limited due to its cost and relative inaccessibility. As *Strimevelis®* is a fresh cell product, patients travel to Milan, Italy (the only treatment centre) for four to six months to undergo treatment.

Although γRV vectors had demonstrated safety and efficacy in ADA-SCID, γRV vectors developed for other PIDs such as X-linked severe combined immunodeficiency (X-SCID) resulted in insertional mutagenesis (discussed later in this review). γRV vectors use strong viral promoters in the LTR sequences to drive transgene expression. By mutating the LTR sequences and by inserting a less powerful mammalian promoter, self-inactivating (SIN)-γRV-vectors were developed (59-61). In addition, a second gene-delivery platform was developed based on a well-known member of the lentivirus (LV) family, the human immunodeficiency virus (HIV). Significant modification of the HIV virus to remove its pathogenic potential and the use of a different packaging envelope, resulted in a gene-delivery vehicle which was able

to insert genetic material into chromatin, but with an integration pattern that had a lower risk of oncogene-activating insertions (62-64). SIN-LV vectors were shown to have an improved safety profile and to be more efficient at gene transfer compared to γRVs (62). To mitigate the risks of insertional mutagenesis in ADA-SCID, alternative vectors have been developed for this disease.

A promising SIN-LV vector has been developed for ADA-SCID which has demonstrated positive results. The latest results from the cohort of 30 patients treated showed 100% survival and just one patient had to restart enzyme replacement therapy and receive a rescue alloHSCT. This cohort was compared to a historical cohort who underwent alloHSCT in which 42% of patients required rescue alloHSCT, restarted enzyme replacement or died (65). Whilst this comparison needs to be interpreted with caution as it was not a randomised clinical trial, these results suggest that HSC GT may offer equivalent if not improved efficacy to alloHSCT. The development of cryopreservation techniques for cellular therapies will undoubtedly improve accessibility of HSC GT products and a clinical trial using this SIN-LV vector for ADA-SCID is evaluating outcomes using cryopreserved transduced cells (NCT03765632, NCT02999984) (30).

Today, over 100 patients have been treated with HSC GT for ADA-SCID on 11 different clinical trials using both γRV and SIN-LV vectors, all of whom are alive to date (30). Although HSC GT has been demonstrated to be effective for ADA-SCID, direct comparisons or randomised studies comparing HSCT-GT with alloHSCT have not been performed. Currently HSC-GT (outside of a clinical trial) is significantly more expensive than alloHSCT although costs are expected to fall with cryopreserved products and improved infrastructure for delivery of cellular therapies. HSC-GT is now considered alongside alloHSCT as a first-line treatment for ADA-SCID in Europe and GT is recommended in preference to alloHSCT from a matched unrelated donor in EBMT treatment guidelines (30, 66). It is expected that a cryopreserved SIN-LV product will move towards market in the near future but where such a product will fit into treatment algorithms (for patients who have a suitable donor for alloHSCT) remains to be seen.

X-SCID

X-SCID is a devastating X-linked disorder in which mutations in the common cytokine receptor gamma chain (IL-2 receptor gene (*IL2RG*) prevent the normal development and function of lymphocytes. Clinically, infants present in the first few months of life with severe infections as a lack of T cells and NK cells result in profound deficits in cellular and humoral immunity. As with ADA-SCID, a definite procedure, either alloHSCT or HSC GT is required to prevent infant mortality (67). Whilst alloHSCT is an effective treatment, patients who lack a sibling donor are at increased risk of GVHD and impaired immune reconstitution (32). Following the initial success in ADA-SCID, similar approaches using γRV vectors were developed to treat X-SCID with a γRV vector entering clinical trials in 1999. Initial results were encouraging with successful immune reconstitution and persistence of gene-marked cells. However, optimism turned to disappointment when 5 out of 20 patients developed acute T lymphoblastic leukaemia early into the trial due to integration of the vector close to proto-oncogenes and subsequent leukemic transformation (68, 69). Further investigation of this phenomenon demonstrated that leukemogenesis was precipitated by unrelated genetic abnormalities but

that integration of the vector close to the LIM domain only 2 (LMO2) caused overexpression of LMO2 in the leukemic clone (69).

Although a theoretical risk of insertional mutagenesis remains, no events have been reported with SIN-LV vectors with to date. These vectors have demonstrated safety and efficacy across many clinical trials in different PIDs. In X-SCID, one SIN-γRV clinical trial and several clinical trials of SIN-LV based HSC GT utilising nonmyeloablative busulfan based conditioning have been reported (33, 59, 67). In the most recently published cohort of 8 infants treated with a SIN-LV vector HSC GT product, with a median follow up of 16.4 months, all demonstrated normal T cell and NK cell numbers (one patient required a boost of gene corrected cells without conditioning) and were developing normally without infections. Vector insertion-site analysis showed polyclonality without clonal dominance (67). There are several clinical trials of HSC GT for X-SCID currently recruiting in the Europe, the US and China (NCT01306019, NCT03601286, NCT04286815) and these are currently the only way in which patients with X-SCID who lack a suitable donor for alloHSCT can access HSC GT. The results of a larger cohort of patients with longer follow up are needed to assess whether HSC-GT for X-SCID is as promising as available results suggest however, if initial observations prove true, commercialisation of a HSC GT product for X-SCID could be expected in the near future. As with ADA-SCID, the place of HSC GT in treatment algorithms will need to be defined following licensing approval with outcomes and costs compared with established alloHSCT protocols.

Artemis SCID and RAG1 deficiency

Less common forms of SCID, but equally devastating, are the syndromes of *Artemis* SCID and recombinase-activating gene 1 (*RAG1*) deficiency. Mutations in the *Artemis* or *RAG1* genes result in severe impairment of T cell function due to V(D)J recombination defects and thus abnormal T cell receptor (TCR) and immunoglobulin receptor rearrangement. In addition to the profound B and T cell dysfunction, *Artemis* SCID patients have cellular radiosensitivity and a predisposition to malignancy (70). AlloHSCT is challenging in *Artemis* SCID due to the sensitivity conferred by the mutation to alkylating chemotherapy or radiotherapy used as preconditioning which is required for adequate immune reconstitution (71). A SIN-LV vector that incorporates the *Artemis* cDNA and the endogenous promoter has been shown to restore T and B cell development following transduction of CD34+ cells from *Artemis* SCID patients and to correct the radiosensitivity in patient fibroblasts (71, 72). The approach is now being assessed in a phase I trial recruiting in the United States (NCT03538899). Development of a GT approach for *RAG1*-deficiency has been slower with challenges in optimising the expression profile of RAG1 in transduced cells (73, 74). This has now been overcome by testing different promoters to optimise expression and the optimised SIN-LV vector that has demonstrated its ability to restore RAG1 expression is expected to enter a phase I trial in the next year (36).

Combined Immunodeficiencies

Wiskott-Aldrich Syndrome (WAS)

WAS is an X-linked PID that results in recurrent infections, severe eczema, microthrombocytopenia, autoimmunity and propensity to develop lymphoid malignancy. Mutations in the *WAS* gene result in defective, reduced or absent production of the Wiskott-Aldrich syndrome protein (WASp) which regulates the polymerisation of actin. Actin is essential for immunological synapse formation, cell migration and cytotoxicity and is required across all haematopoietic lineages (75). WAS results in progressive morbidity and early mortality and definitive treatment is increasingly offered to all patients following diagnosis. AlloHSCT is curative and whilst outcomes are good with overall survival rates at two years of over 85%, outcomes are significantly worse in patients over five years of age (38). The first human trial of γRV-based HSC GT for WAS was initiated in 2006 but similarly to the adverse events seen in the HSC GT X-SCID trials, leukemogenesis occurred in a majority (7/9) of the patients treated (76).Transgene integration around proto-oncogenic loci (LMO2/MDS1/EVI) was found to be driver of leukemogenesis prompting the development of alternative vectors (76, 77).

A SIN-LV vector was subsequently developed between groups in London and Pairs, that used a fragment of the endogenous *WAS* gene promoter to drive gene expression (78, 79). A clinical trial of this vector (using fludarabine and busulfan conditioning) has reported encouraging results with good immune reconstitution, WASp expression across all lineages, maintenance of gene-marked cells and a majority of patients have been able to cease immunoglobulin replacement therapy (79). This trial included the first adult (30 years old) HSC GT patient (16). A similar SIN-LV vector has been developed in parallel by an Italian group which has also demonstrated encouraging results (39). Whilst SIN-LV HSC GT approaches appear safe and have resulted in clinical improvement, WAS poses some challenges which have not been completely overcome. Platelet counts remained below the normal level, although they increased from pre-treatment values (WASp positive platelets increased from a median of 19.1% to 76.6% in the Milan trial), which is hypothesised to be due to the lack of selective advantage conferred by WASP expression in platelet precursors (39, 80). Although numbers are still small, the absence of mortality amongst patients treated with HSC GT for WAS and the more limited toxicities associated with an autologous approach makes HSC GT an attractive alternative to alloHSCT for patients (80). In the near future, should a HSC GT product for WAS gain licensing approval, it should be considered for patients with limited donor availability. HSC GT may have a greater advantage over alloHSCT in older patients with WAS who have a higher risk of TRM and GvHD with alloHSCT (81). The ability to offer HSC GT as a first line treatment for WAS to all patients will depend on longer term outcomes, economic considerations and results in relation to current alloHSCT approaches.

Disorders of Phagocyte Number and Function

Chronic Granulomatous Disease (CGD)

CGD results from mutations in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex which impairs production of reactive oxygen species and thus results in functional impairment of phagocytes; neutrophils, monocytes and macrophages. Clinically, manifestations of CGD include life-threatening bacterial and fungal infections and hyperinflammation that often manifests as treatment-resistant colitis (82). The commoner X-linked variant accounts for two-thirds of cases and results from mutations in *CYBB* gene which encodes the gp91phox subunit of NADPH-oxidase. The rarer autosomal recessive form of CGD accounts for the remaining third of cases. Contrary to SCID variants, CGD has a variable clinical phenotype and can present later in childhood or adolescence (83). Long-term registry studies report that 50-55% of patients survive to their fourth decade with supportive care however, significant disease-related morbidity frequently results in severe impairment of quality of life (84). AlloHSCT is curative and excellent survival outcomes have been achieved with RIC conditioning (13). Increasingly, definitive therapy is recommended as early as possible to preserve quality of life and prevent premature mortality although donor availability and risks of an allogeneic approach in patients with severe disease-related co-morbidities can preclude alloHSCT (12-14, 85).

As with X-SCID and WAS, HSC GT was first attempted for CGD with γRV vectors. In order to correct the clinical phenotype sufficient gene marking is required in the myeloid lineage. Early trials in 1995 and 1997 which did not use conditioning, failed to achieve adequate myeloid gene marking and engraftment and persistence was poor (86). Improved gene marking was achieved using a γRV vector derived from a murine spleen focus-forming virus; however, this trial was also complicated by insertional mutagenesis similar to that seen in X-SCID and WAS trials. Potent enhancer element-driven clonal expansion following gene insertion in the *EVI1/MDS1* gene complex led to the development of monosomy 7 derived MDS (87, 88). In addition to their integration adjacent to proto-oncogenes, it was found that the LTR promoter elements in the γRV CGD vector were being methylated leading to silencing of the transgene (88, 89).

As with other PIDs, safer SIN-LV vectors have been developed. Myeloid gene marking was improved by the use of a novel chimeric myeloid promoter to drive the codon optimised *CYBB* cDNA in a SIN-LV vector (90, 91). This vector has entered clinically trials in the United States and Europe and recently published promising results with 16-46% of patient neutrophils displaying oxidase-positivity at six months (41). Notably the majority of patients in this cohort (6/9) were aged over 18 years demonstrating that immune reconstitution can be achieved in older patients with autologous GT (41). A parallel SIN-LV approach for autosomal recessive CGD (p47 deficiency) is also in clinical development (42). HSC GT will need to demonstrate long term efficacy in order to supplant alloHSCT as the treatment of choice for patients with a suitable donor. However, the additional costs of HSC GT are more easily justifiable in older patients with CGD in whom significant disease-related co-morbidities can make allogeneic procedures extremely high risk.

Leukocyte Adhesion Defect Type 1 (LAD-1)

Leukocyte adhesion defect type 1 (LAD-1). LAD-1 is a disorder of phagocyte function that results from mutations in the *ITGB2* gene leading to defective or absent β-integrin molecules (CD11a and CD18) leading to impaired migration and adhesion of leucocytes. The disease usually presents with deep tissue infections and delayed wound healing in infancy (92). AlloHSCT is curative with overall survival rates of around 75% (92). A pre-clinical proof-of-concept demonstrated that SIN-LV vectors conferring ubiquitous or preferential expression of CD18 were able to restore membrane β-integrin expression in patient CD34-derived granulocytes cells *in vitro* and *in vivo* in a mouse model ($CD18^{HYP}$) of LAD-1 (45). Phase I studies of this HSC transduced with this LV vector with prior busulfan conditioning are currently recruiting in Madrid, London and Los Angeles (NCT03825783, NCT03812263). Given that alloSCT appears to have a higher mortality and rejection risk compared to other PIDs which require alloHSCT in infancy, HSC GT may prove to be a safer treatment option.

Diseases of Immune Dysregulation

Familial hemophagocytic lymphohistiocytosis (FHL)

Haematologists will be all too familiar with life-threatening haemophagocytic lymphohistiocytosis (HLH) characterised by hyperinflammation, uncontrolled immune activation, splenomegaly and cytopenias (93). HLH can be driven by a secondary cause such as malignancy, infection or autoimmunity but can also result from one of the genetic mutations (primary HLH) of Familial hemophagocytic lymphohistiocytosis (FHL). Whilst presentation is childhood is more common, FHL can present in adulthood. FHL results from mutations that cause defective cytotoxic T cell and Natural Killer (NK) cell function including autosomal recessive mutations in Perforin (*PRF1*), MUNC 13-4 (*UNC13D*) and syntaxin 11 (*STX11*) of which *PRF1* mutations are the most common.

Patients with a detectable genetic mutation or relapsed refractory HLH in the absence of a genetic diagnosis should receive alloHSCT if possible (94, 95). Suppression of the inflammation prior to alloHSCT is required and achieving disease control can be challenging. Glucocorticoids, etoposide with or without cyclosporine is the current standard of care but these toxic regimens fail to achieve adequate disease control in up to 40% of patients (96).. Interferon gamma (IFNγ) has a key role in the pathogenesis of HLH with levels correlating with active disease (97). The anti-IFNγ monoclonal antibody, emapalumab, has also recently demonstrated safety and efficacy in a fragile population of patients with primary HLH leading to its regulatory approval as a second line therapy in the US (98). Other strategies to improve remission and allow progress to HSCT are also being investigated including alemtuzumab and JAK inhibitors (99-104). Recently, alemtuzumab together with corticosteroids and cyclosporine has demonstrated favourable safety and efficacy profiles in children with primary HLH (91.6% survived to alloHSCT) (105).

HSC GT and T cell GT have been proposed as another therapeutic strategy to treat primary HLH although a unique approach is required for each causative genetic defect. Correction of a murine model of FHL ($Prf^{-/-}$) has been demonstrated with adoptive transfer experiments

with T cells and HSCs transduced with SIN-γRV, SIN αRV or LV vectors incorporating the Perforin cDNA (47, 106, 107). Clinical trials in humans are needed to assess whether a T cell strategy would provide long-term efficacy or whether transient clinical improvement would offer a bridge to a definitive HSC based therapy. Pre-clinical validation of HSC GT for perforin defects has also been published, with HSCs transduced with a LV vector encoding the human perforin gene under the influence of a phosphoglycerate kinase (PGK) promoter resulting in differentiation of normal T and NK cells with adequate perforin expression in mouse models of the disease (107). Perforin GT has not entered clinical trials at the time of writing although a study is planned (Booth, personal communication). A SIN-LV GT approach is also under development for another common cause of FHL, Munc 13-4 defects, and again both HSC and T cell approaches have been considered (107-111).

X-linked lymphoproliferative disease 1 (XLP1)

X-linked lymphoproliferative disease 1 (XLP1) results from mutations in the *SH2D1A* gene that causes defects in the intracellular adaptor protein, SLAM-associated protein (SAP), essential for T-cell and NK-mediated cytotoxicity and T follicular helper cell function (112). Clinically XLP1 manifests as immune dysregulation with HLH and the development of lymphoma (51). AlloHSCT is the only curative therapy for XLP1 however, survival outcomes are worse if active disease is present at time of transplant (51). Pre-clinical work has demonstrated that transduction of T cells with a SIN-LV vector incorporating *SAP* cDNA can engraft and persist in *SAP* deficient mice and both improved cytotoxicity and humoral recovery were observed when T-cells from XLP1 patients were transduced with this vector (52). This T cell GT approach will be assessed in human clinical trial that is in the planning stages (Booth personal communication).

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome

Regulatory T cells (T_{regs}) are essential for immune regulation. T_{regs} control the immune response to self-antigens, preventing autoimmunity. Defects in the transcription factor FoxP3 which is required for the normal development and function of T_{regs} results in IPEX syndrome and the clinical manifestations of enteropathy, type I diabetes mellitus and eczema. Long-term immunosuppression can be used for disease control and alloHSCT is potentially curative but can be affected by immune-mediated complications (48). A T-cell approach, while feasible may not generate adequate numbers of T_{regs} to improve the clinical phenotype (113). A HSC GT approach is more challenging as FoxP3 expression in HSCs prevents proliferation and differentiation of the stem cells. In order to prevent ubiquitous FoxP3 expression in HSCs a SIN-LV vector has been designed that incorporates the endogenous FoxP3 promoter and some regulatory elements, which was shown to induce lineage-specific expression in the progeny of transduced HSCs (49, 113, 114). This approach is expected to enter clinical trials in the near future.

Recent and Future Developments

HSC GT for adolescents and adults with PID

An important advance that has occurred in the last five years is the demonstration that adolescent and adult patients can be successfully treated with HSC GT. Whilst most forms of SCID are fatal in infancy without a definitive treatment, survival to adulthood with good supportive care in non-SCID PIDs is possible. Indeed, many PIDs can present later in childhood or early adulthood or an initial mild phenotype can mean that patients present in their second and third decade with serious life-threatening complications (4). Historically, outcomes for alloHSCT for older patients with PID have been dismal however, improved outcomes have been recently demonstrated with reduced-intensity conditioning regimens (12). Survival for adults with PID undergoing alloHSCT is still inferior to those seen in younger patients and an autologous approach for adults, particularly those with disease related co-morbidities, would carry significantly less risk.

Whilst HSC GT has been demonstrated to be curative in infants and children there was concern that a mature immune system may not be able to generate a full T-cell repertoire using an autologous GT approach. In 2017, the first report of a 30 year old adult with WAS, successfully treated with autologous GT was published and suggested that these fears were unfounded (16). The patient did not have a suitable donor for alloHSCT and had severe disease-related comorbidities that would preclude a haploidentical alloHSCT. Following engraftment and expansion of a polyclonal pool of gene-corrected T-cells the patient had sustained gene marking in myeloid and B-cell lineages (16). Adult patients have since been recruited onto other HSC GT trials. Patients aged over 18 made up a majority (6/9) of the subjects on the recently reported phase I trial of LV-based GT for X-linked CGD (41). Due to the prevalence of disease-related comorbidities in older patients with PID, it may be that adult patients will benefit significantly from the development of autologous GT approaches.

Gene Editing

Gene editing refers to a group of technologies which enable precise editing of a DNA sequence. In contrast to virus-mediated gene transfer techniques where the transgene inserts semi-randomly into the genome at a site distant from the endogenous mutated gene, gene editing repairs or replaces the mutation *in frame* within the coding sequence. Gene editing has two key steps, the creation of a double strand DNA break and the repair of this break (see figure 2). The creation of the double strand break can be initiated by several designer DNA endonucleases. Transcription activator-like effector nuclease (TALENs), zinc-finger nucleases and meganucleases can all perform a similar function, however in 2012 the development of the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats associated with Cas9 endonuclease) RNA-based system has spurred the development of gene editing strategies due to its relative ease of use compared to other technologies (25, 115). The CRISPR-Cas9 system enables the same high specificity of sequence targeting of the other endonuclease systems, yet its application is easier due to the guidance of the Cas9 endonuclease to its target site being governed by Watson-Crick base pairing (116). In addition, due to rapid uptake of the technology by the research community, reagents are readily and widely available.

The CRISPR-Cas9 system requires two components, the Cas9 nuclease and a guide RNA (gRNA) (116). After the gRNA binds to its target sequence of DNA the Cas9 enzyme undergoes a conformational change which then causes it to cleave DNA at a precise point (see figure 2). In the absence of a repair template, DNA will preferentially repair by the process of non-homologous end joining (NHEJ) (117). NHEJ is used in all cells to repair DNA breaks that occur as part of normal cellular aging, due to DNA-damaging insults, as well as in normal T cell development to create diversity in T cell receptor (TCR) and immunoglobulin genes (118). NHEJ is error prone and can create a small insertion or deletion (indel) at the site of the double-strand break. NHEJ can be used for therapeutically useful applications for example knocking out a gene or to disrupt the BCL11A erythroid enhancer to promote γ-globulin (fetal haemoglobin) production in β-thalassaemia (119).

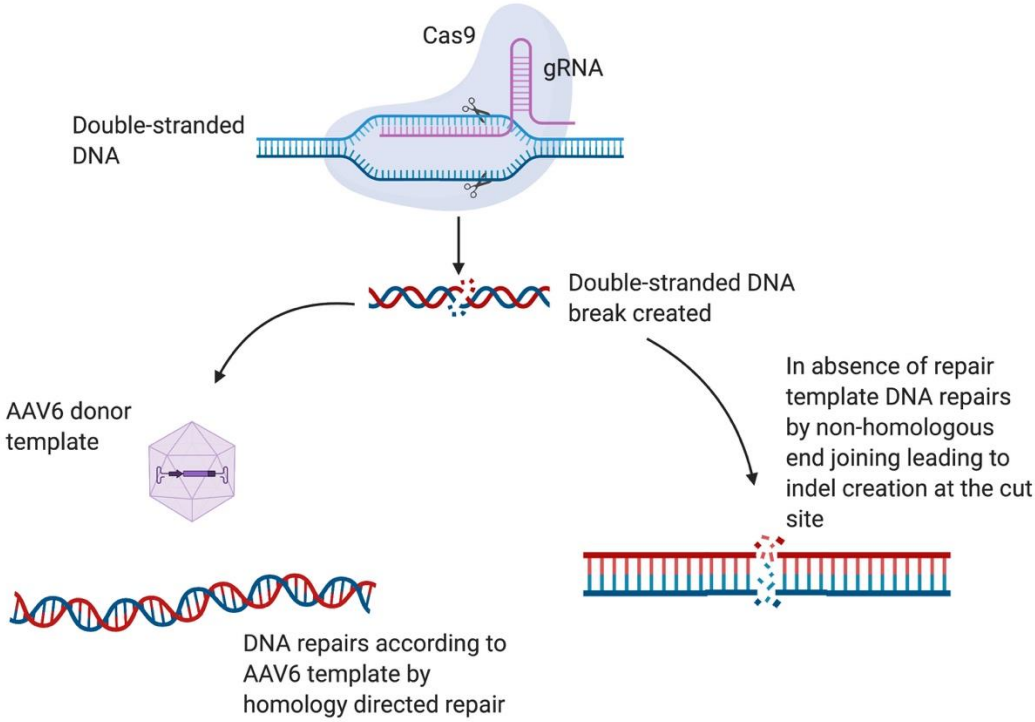


Figure 2: Schematic diagram demonstrating the mechanism by which CRISPR/Cas9 enables editing of DNA. In the absence of a repair template NHEJ occurs resulting in indel formation and gene knock-out (right). In the presence of a DNA repair template NHEJ can occur which enables the repair of a DNA sequence or the insertion of new genetic material at the target site. Created with BioRender.com

The other main mechanism of DNA repair is homology directed repair (HDR). In the presence of a donor template (delivered by viral transduction such as adeno-associated virus (AAV) or double or single-stranded oligonucleotides), that contains homology arms which flank the target region, new DNA that is complementary to the template can be synthesised (25, 120). Gene editing has several potential advantages over viral gene addition (see table 2). There is

less risk of insertional mutagenesis as a precise target site in the genome undergoes editing, although off target cutting is a possible risk. The editing occurs at the genetic locus of the transgene of interest thus gene expression remains under the influence of the endogenous gene control machinery. Editing at the endogenous locus is hypothesised to enable more 'physiological' expression and may improve transgene expression across different haematopoietic lineages. Gene editing may also be applied to a wider range of PIDs for example dominant negative mutations or diseases that result from haploinsufficiency. Gene editing also carries its own unique risks and in particular there is concern that off-target DNA breaks could result in damaging mutations elsewhere in the genome.

	Allogeneic HSCT	**Autologous HSC Gene Therapy (using viral gene addition)**	**Autologous HSC Gene Therapy (using gene editing)**
Donor	Requires suitably matched donor. Sibling donors preferred (although need to exclude that unaffected by same genetic mutation).	No need for suitable donor.	No need for suitable donor.
Conditioning	RIC conditioning can now be used successfully for PID alloSCT.	Some conditioning needed (RIC or just low dose busulfan depending on disease).	Some conditioning needed (RIC or just low dose busulfan depending on disease).
Experience of procedure and access to treatment	Over 50 years' experience. Good data and risks well known. Widely available in specialist transplant centres.	Over 25 years' experience in some disease settings. Not widely available, can only be performed in select centres. Only one product for PID with marketing approval (*Strimvelis* for ADA-SCID).	First in-human trials of gene editing therapeutics have started (e.g. haemoglobinopathies) but no clinical trials for PID yet. Trials expected to open in a few selected centres in the near future.
Efficacy	• OS in SCID cohorts >95%. • OS for other PIDs 75-95%. • Inferior outcomes in older patients (~85% in adult cohorts).	• Over 80 patients treated (across all trials) and 100% survival and no genotoxic events for ADA-SCID (10-20% re-start enzyme replacement or had alloSCT) (31). • LV GT for X-SCID has minimal toxicity and reconstitutes function T and B cells (normalises NK counts) (67). • 91% survival in 34 patients treated on GT trials for WAS with sustained gene marking, variable platelet count recovery (121). • 2/9 patients died of pre-existing co-morbidities on CGD LV HSC GT trial (41).	• No trials in PID to date. • Early safety data from haemoglobinopathy trials promising. • Multilineage gene marking may be improved in some settings (e.g. improved platelet recovery in WAS seen in pre-clinical data) (40).
Significant Risks	• GvHD • Graft rejection • Graft failure	• Risk of insertional mutagenesis (has not been observed with LV vectors). • Transgene is not under the endogenous gene control machinery.	• Off-target ds breaks. • Long term safety unknown. • Low efficiency of homology. directed repair may reduce numbers of corrected cells.

Table 2: Comparison of alloHSCT, HSC GT using viral gene addition and HSC GT using gene editing for PID.

The pace of development of gene editing technologies has been remarkable and since its discovery in 2012, CRISPR-based therapeutics have already entered the clinic. At the time of writing there are 39 active clinical trials using CRISPR/Cas9 edited cellular therapies worldwide, including trials to treat genetic diseases such as beta thalassaemia (NCT03655678) and sickle cell anaemia (NCT03745287). Early safety data from the first two patients treated in the aforementioned trials appears promising, although clearly much longer follow up in a larger cohort is needed. Whilst there are no clinical trials of gene editing therapeutic approaches for PID open currently, there are several promising proof-of-principle studies, paving the way for human trials in the near future. There are several gene editing strategies which can be employed depending on the mutational landscape of the disease (outlined in table 3). Studies have been published which demonstrate promising gene editing approaches for SCID-X1, WAS, X-CGD, hyper IgM syndrome, IPEX syndrome and X-linked agammaglobulinaemia (34, 40, 50, 122-124).

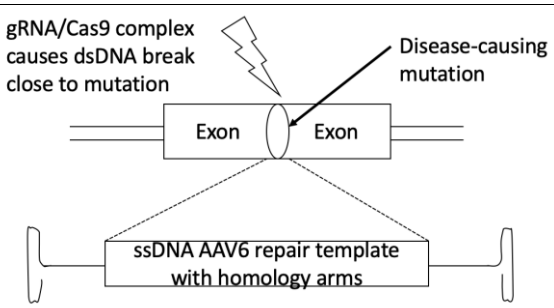
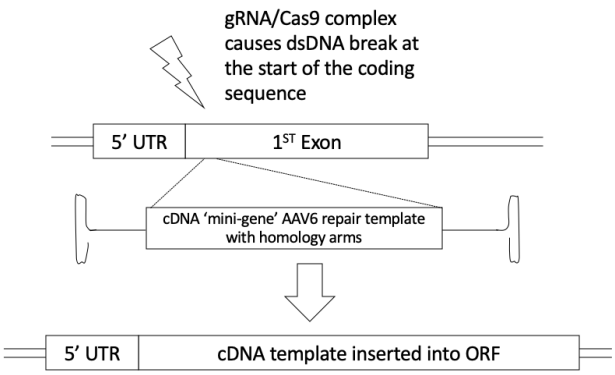
Editing Strategy	**Mutational landscape**	**Example**	**Illustration**
Direct repair of disease-causing mutation.	Requires a single mutation to be responsible for the majority of cases.	Direct repair of *CYBB* 676 locus in $CD34^{+}$ cells for the treatment of X-CGD (34).	
Insertion of a cDNA cassette into the endogenous locus of a particular gene.	Heterogenous mutational landscape	Insertion of *WAS* cDNA into the endogenous locus (40)	

Table 3: Outline of the different gene editing approaches for PIDs.

The pre-clinical data in WAS is an excellent example that highlights the potential benefits of gene editing techniques over viral gene addition strategies. As previously outlined, HSC GT using stem cells transduced with a SIN-LV vector has resulted in substantial clinical improvement in patients treated (39). However, correction across different lineages varied, with excellent T lymphocyte expression but less robust expression in platelets and variable

correction of thrombocytopaenia. A promising pre-clinical study recently reported the successful application of a CRISPR/Cas9/AAV6 gene editing approach which was able to insert the *WAS* cDNA into the endogenous gene locus (40). Following gene editing of HSCs, correction of *WAS* and physiological levels of WASp were observed in mature haematopoietic cells including T-cells, B-cells, platelets and macrophages. In addition, following lentiviral transduction of stem cells there was a reduction in the multipotent progenitor cells (MPPs) population, suggesting a loss of multipotency which was not seen when gene editing was performed at the *WAS* locus (40).

Gene editing also enables correction of disorders where a viral gene addition strategy may result in adverse effects. An excellent example of this hyper-IgM syndrome, where it has been demonstrated in mouse models of the disorder that whilst retroviral vectors can correct the immune defect, unregulated gene expression results in abnormal lymphoproliferation (125). In-frame targeted gene addition, that utilises the endogenous promoter and gene control machinery has been demonstrated to successfully correct the immune defect in human T cells and haematopoietic stem cells (122, 123). Gene editing strategies are also in development for other disorders where gene-addition and unregulated gene expression could be problematic such as XLP, *CTLA4* deficiency and *STAT1* gain-of-function mutations (Booth/Morris, personal communication).

Although the long-term safety of gene editing in patients still needs to be ascertained, the technology appears promising and the first clinical trials of a gene editing therapies for PID are expected to open in the next few years.

Non-genotoxic conditioning

Much of the toxicity associated with HSC GT results from the conditioning regimen. The ability to avoid DNA-damaging chemo and/or radiotherapy and their subsequent early and late side effects would be a significant therapeutic advance. Antibody-based conditioning agents that are able to deplete HSCs are in the advanced stages of clinical development and if they prove to be effective would further broaden the appeal of HSC GT for PID. Several different targets are being assessed for their efficacy, with AMG191 which targets CD117 (c-Kit), a tyrosine kinase receptor expressed on the surface of HSCs, at the most advanced stage of clinical development (126). AMG191 has been shown to safely deplete HSCs in non-human primate models (127). A phase I dose-escalated clinical trial in humans (NCT02963064) has been initiated in the context of alloHSCT for SCID and early results show evidence of sustained engraftment of multipotent donor HSCs (126). It remains to be seen whether non-genotoxic conditioning agents such as AMG191 will be successful in the non-SCID setting however if the *in vitro* and animal work holds true in human clinical trials, these novel agents may permit HSC GT without alkylating agents or irradiation.

Conclusions

It is an exciting time in the field of HSC GT for PID. Whilst significant challenges remain, it is likely that GT will become a treatment option or even standard-of-care for specific PIDs in the

near future. The role of HSC GT in treatment algorithms in diseases where outcomes from alloHSCT are excellent (such as ADA-SCID) will need to be determined although if financial and logistical challenges are overcome an autologous approach may be preferred in the first instance by patients and their families. For older patients or in PIDs for which alloHSCT carries a high TRM such as Artemis SCID and XIAP, HSC GT may offer a cure for patients who would otherwise have limited treatment options. At the current time, the high cost and limited availability of HSC GT compared to alloHSCT precludes the widespread adoption of GT. However, infrastructure for the manufacture of adoptive cellular therapies is being rapidly developed in many countries and this may start to reduce costs and improve availability. Large scale, serum-free, good manufacturing practice (GMP) compliant automated systems are now available for virus manufacture and cell transduction. Such automated platforms reduce the risk of contamination, minimise labour costs and also help lower the cost of manufacture (128). Gene editing technologies and non-genotoxic conditioning agents may further improve the efficacy, broaden the scope and reduce the risks associated with HSC GT.

As awareness of PIDs increases amongst the immunology and haematology communities we are likely to identify more patients who have an inborn error of immunity driving their clinical phenotype. Whilst alloHSCT remains a suitable treatment option for most patients affected by PIDs, the development of HSC GT approaches may enable us in the future, to offer a safe effective definitive therapy that abrogates the immunological complications of alloHSCT.

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