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Clinical Applications of Gene Therapy for Primary Immunodeficiencies

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Abstract

Primary immunodeficiencies (PIDs) have represented a paradigmatic model for successes and pitfalls of hematopoietic stem cells gene therapy. First clinical trials performed with gamma retroviral vectors (γ-RV) for adenosine deaminase severe combined immunodeficiency (ADA-SCID), X-linked SCID (SCID-X1), and Wiskott–Aldrich syndrome (WAS) showed that gene therapy is a valid therapeutic option in patients lacking an HLA-identical donor. No insertional mutagenesis events have been observed in more than 40 ADA-SCID patients treated so far in the context of different clinical trials worldwide, suggesting a favorable risk–benefit ratio for this disease. On the other hand, the occurrence of insertional oncogenesis in SCID-X1, WAS, and chronic granulomatous disease (CGD) RV clinical trials prompted the development of safer vector construct based on self-inactivating (SIN) retroviral or lentiviral vectors (LVs). Here we present the recent results of LV-mediated gene therapy for WAS showing stable multilineage engraftment leading to hematological and immunological improvement, and discuss the differences with respect to the WAS RV trial. We also describe recent clinical results of SCID-X1 gene therapy with SIN γ-RV and the perspectives of targeted genome editing techniques, following early preclinical studies showing promising results in terms of specificity of gene correction. Finally, we provide an overview of the gene therapy approaches for other PIDs and discuss its prospects in relation to the evolving arena of allogeneic transplant.

Introduction

Primary immunodeficiencies (PIDs) represent a heterogeneous group of monogenic conditions determined by altered immune responses of innate and/or adaptive immunity.[1] More than 260 disorders have been identified, resulting from mutations in over 300 genes.[2,3] Their number is rapidly increasing thanks to next-generation sequencing technologies and increased clinical awareness.[2]

The incidence of PIDs ranges from 1 in 600 to 1 in 500,000 live newborns, depending upon the specific disorder.[4,5] Patients with PIDs display phenotypes that can range from being asymptomatic to manifestation of life-threatening conditions (e.g., various forms of severe combined immunodeficiency, SCID). With new information on genes affecting the immune system and discovery of new pathogenic mutations and molecular mechanisms, different clinical presentations are attributed to gene defects that, in the past, appeared to have a traditional presentation only.[2,6]

Additionally, an increasing number of syndromes are also characterized by immune dysregulation with autoimmunity and susceptibility to lymphoreticular malignancy.[5,7,8]

While differing in clinical severity, early diagnosis and treatment remain a mainstay for all forms of PIDs to prevent organ damage and life-threatening infections and to improve prognosis and quality of life.[6,9] Major efforts have recently been undertaken to develop methods for detection of PID in the neonatal period; in particular, a triplex RT-qPCR measuring the levels of TRECs and KRECs has been shown to provide a suitable screening for the vast majority of severe immunodeficiency diseases characterized by T- or B-lymphopenia in newborns.[6] Universal newborn screening in the United States has helped to establish the true incidence of SCID in California (1 in 66,250 live births) and has led to the improvement of survival outcome.[9] Recently, tandem mass spectrometry for analysis of metabolites from dried blood spots has been proposed as an easy and cheap method for adenosine deaminase (ADA) SCID screening.[10,11]

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Bone marrow transplantation (BMT) still remains the definitive cure for most of the PIDs, and the outcomes of patients treated in European centers are improving over time.[12,13] Survival is excellent in HLA genoidentical donor setting and is progressively increasing in other settings thanks to the improvement in conditioning regimens, prophylaxis and treatment of infectious complications, GvHD prevention, stem cells selection and manipulation, and choice of unrelated donors.[13–17]

In the last 15 years, gene therapy (GT) has been successfully implemented for the treatment of PID patients who lacked a suitable donor. In some cases, efficacy of gene therapy has been counterbalanced by the occurrence of insertional oncogenesis. The understanding of the molecular events that led to oncogenesis and improved vector technology allowed to progress with safer gene therapy approaches for PID.

Here we review the most recent results on clinical trials for X-linked SCID (SCID-X1), ADA-SCID, Wiskott–Aldrich syndrome (WAS), and chronic granulomatous disease (CGD) and discuss perspectives for new technologies and other diseases.

Gene Therapy for SCID-X1

X-linked SCID is actually the most common form of SCID, accounting for 40–50% of SCID cases reported worldwide.[5] Mutations in the *IL2RG* gene are leading to defective expression of the common gammachain (γc), a subunit shared by a host of cytokine receptors, including interleukin (IL)-2, 4, 7, 9, 15, and 21 receptor complexes, which play a vital role in lymphocyte development and function.[5] As a consequence, SCID-X1 patients present profound immunological defects caused by low numbers or complete absence of T and NK cells, and presence of nonfunctional B-cells.[18] Death from community-acquired or opportunistic infections usually occurs before 1 year of age unless allogeneic hematopoietic stem cell transplantation is performed.[19]

While allogeneic transplantation from an HLA-identical donor has a high survival rate, persistent defects in humoral or cellular functions have been reported for some patients, resulting in partial immune recovery, autoimmunity, and/or retarded growth.[19,20] On the other hand, transplantation from mismatched related, matched unrelated, or umbilical cord donors in patients with ongoing infection is associated with lower survival rates, often partial chimerism of hematopoietic lineages with persistent impairment of humoral immune function,[21] and higher rates of complications as graft-versus-host disease.[12–14,21]

SCID-X1 was thought to be the most accurate model for assessing GT, because spontaneous reversion of the mutation in the γc-encoding *IL2RG* gene led to restoration of immunological competence, suggesting that transduced lymphocyte progenitors could carry a selective advantage over their nontransduced counterparts.[5,22]

Between 1999 and 2006, twenty subjects with SCID-X1 lacking HLA-identical bone marrow donors have been treated in two trials, conducted in Paris and London. The treatment consisted of an infusion of autologous CD34+ bone marrow cells transduced with a first-generation Moloney murine leukemia virus vector expressing the γc complementary DNA (MFG-γc) and containing duplicated viral enhancer sequences within the long terminal repeats (LTRs). Gene therapy resulted in correction of the immunodeficiency, with polyclonal and functional T-lymphocytes in 19/20 patients.[3,23–26]

Engraftment and correction of NK and B cells was lower, likely because patients did not receive conditioning. Immunoglobulin replacement treatment was stopped in 11/20 patients, allowing most patients to live a normal life.[3,23] An 85% survival rate was observed with a median follow-up of 13 years, similar to the results obtained with matched-sibling donor hematopoietic stem cell transplantation (HSCT),[19] demonstrating that gene therapy can be curative for X-SCID with long-lasting (10 years) beneficial effects.[19] Four patients in the French trial and one patient in the British cohort have developed T-cell leukemia[24] between 2 and 5 years after GT: four of them have been into remission after conventional chemotherapy, in one case followed by matched unrelated hematopoietic cell transplantation (HCT), and remain in long-term remission,[25,26] while the remaining patient has died from chemotherapy-refractory leukemia. In all cases, the adverse event was the result of insertional oncogenesis caused by aberrant expression of the LMO2 (LIM domain only-2) or CCND2 (cyclin D2) oncogenes induced by the integration of the γc retroviral vector (RV) in the proximity of the gene regulatory regions.[19] Second genome alterations were found in all cases and probably accounted for the advent of overt leukemia,[3] favored by the selective advantage conferred to them by the concomitant expression of the γc gene.[23] The occurrence of these serious complications prompted discontinuation of these trials.[24]

A further trial was started at the NIH in 2003 as a treatment option for older X-SCID patients for whom HCT was not successful. Three patients (11, 10, and 14 years old) were treated with granulocyte colony stimulating factor (G-CSF)-mobilized peripheral blood CD34+cells transduced with γ-RV: T-cell numbers and function improved only in one subject, the youngest, and no immunological improvement was found in the other two.[23]

To improve safety while maintaining the efficacy profile for X-SCID gene therapy, a self-inactivating (SIN) γ-RV with deleted Moloney murine leukemia virus LTR U3 enhancer was exploited, expressing the IL2RG complementary DNA from the eukaryotic human elongation factor 1α (EF1α) short promoter, and having shown to be less mutagenic *in vitro*, although effective in the mouse model of X-SCID (enhancer-deleted SIN-γc).[27,28] The interim results of the first nine patients treated in parallel phase 1/2 trials conducted in London, Paris, Boston, Cincinnati, and Los Angeles have been recently published,[19] and the trial is still recruiting patients (Table 1). SCID-X1 children were enrolled if an HLA-identical sibling donor was not available or in the case of severe ongoing, therapy-resistant infections. Eight out of nine treated patients survived, while a preexisting disseminated adenovirus infection was fatal to one patient 4 months after GT, before the full reconstitution of the T-cell compartment. Up to 48 months of follow-up, immune reconstitution of T-cells occurred in the other 7 patients and was comparable to that observed in the previous trials conducted in Paris and London. Importantly, integration analysis showed a polyclonal integration profile with reduced numbers of clones near known lymphoid proto-oncogenes and genes implicated in serious adverse events in previous GT trials.[19]

A new approach based on the use of SIN lentiviral vector (LV; CL20-i4-EF1α-hγc-OPT) expressing a γc gene has

Table 1. Ongoing Gene Therapy Clinical Trials for Primary Immunodeficiency

Disease	*Country*	*Sponsor*	*Vector*	*Outcome*	*Reference*	*Clinical Trials.gov ref no.*
SCID-X1	France UK US	Assistance Publique—Hôpitaux de Paris, Great Ormond Street Hospital, Children's Hospital Boston	SIN γ-RV	8/9 patients alive, immune recovery with clinical benefit	Hacein-Bey-Abina et al.[19]	NCT01410019 NCT01175239 NCT01129544
SCID-X1	US	St. Jude Children's Research Hospital, National Institute of Allergy and Infectious Diseases	SIN-LV	Two young adult patients, improved IgG production	De Ravin et al.[30]	NCT01512888 NCT01306019
ADA-SCID	Italy	GlaxoSmithKline	γ-RV	15/18 patients off ERT, clinical benefit	Aiuti et al.,[54] Cicalese et al.[117]	NCT00598481
ADA- SCID	UK	Great Ormond Street Hospital	γ-RV	4/6 patients off ERT, clinical benefit	Gaspar et al.[55]	NCT01279720
ADA- SCID	US	Donald B. Kohn, UCLA	γ-RV	9/10 patients off ERT, clinical benefit	Candotti et al.,[56] Carbonaro Sarracino et al.[118]	NCT00794508
ADA-SCID	UK US	Great Ormond Street Hospital; Donald B. Kohn, UCLA	SIN-LV	5 patients, immune and metabolic recovery	Gaspar et al.[119]	NCT01380990 NCT01852071
WAS	Italy	IRCCS San Raffaele	SIN-LV	6 patients, immune, hematological and clinical improvement	Aiuti et al.,[87] Scaramuzza et al.[120]	NCT01515462
WAS	France UK	Genethon	SIN-LV	France: 4/5 patients alive, clinical improvement	Bosticardo et al.[89]	NCT01347346 NCT01347242
WAS	US	Children's Hospital Boston	SIN-LV	2 patients, immune and hematological improvement	Williams[121]	NCT01410825
CGD	Germany	Hubert Serve, Johann Wolfgang Goethe University Hospitals	SIN γ-RV			NCT01906541
CGD	Germany Switzerland UK US	Genethon UCLA	SIN-LV		Kaufmann et al.[109]	NCT01855685 NCT02234934

ADA, adenosine deaminase; CGD, chronic granulomatous disease; ERT, enzyme replacement therapy; LV, lentiviral; RV, retroviral vector; SCID, severe combined immunodeficiency; SIN, self-inactivating; WAS, Wiskott–Aldrich syndrome.

Studies are classified as active and ongoing based on information retrieved from ClinicalTrials.gov. Updated information on recruitment status can be found on ClinicalTrials.gov.

been developed by Sorrentino and colleagues[29] and is used in a two-site clinical trial. Typical X-SCID patients will be enrolled at the St. Jude Children's Research Center in Memphis, while atypical children and adolescents between 2 and 20 years of age are treated at NIH (Table 1). The latter arm of the trial uses nonmyeloablative conditioning with a total busulfan dose of 6 mg/kg/body weight to improve the efficacy of engraftment of gene-corrected cells.[30] Preliminary results in two young adults after 15 and 9 months from GT show restoration of Ig production and B-cell function, increasing gene-marked NK cells, and clinical improvement.[30]

Gene Therapy for ADA-SCID

ADA-SCID, caused by mutations in the *ADA* gene impairing ADA activity, stability, and survival and leading to accumulation of toxic metabolites in plasma, red blood cells, and tissues, represents the second most frequent form of SCIDs, accounting for 15–20% of all cases of severe combined immunodeficiencies.[31,32] In its typical early severe onset form, it is usually fatal in the first year of life.[33] Apart from the profound lymphopenia (T, B, and NK) and the absence of cellular and humoral immune function,[34] nonimmunological alterations as manifestation of the metabolic organ damage have been described.[35,36]

HLA-matched sibling donor (MSD) or family donor (MFD) SCT is the gold standard in ADA-SCID therapy and is associated to excellent overall survival (86% for MSD and 83% for MFD). Data from a large cohort of ADA-SCID patients transplanted with alternative donors over 20 years clearly show the importance of donor matching in improving outcome, with 67% overall survival (OS) in HLA-matched unrelated donors (MUDs) (67%) and 43% and 29% OS in haplo and mismatched unrelated donor SCT, respectively.[37,38] Moreover, the metabolic nature of the disease and the need for conditioning regimens make mismatched transplantation for this form of SCID more difficult to manage than other forms, even for the associated risks.[21,32] Beyond this, once patients survive the procedure and engraft donor cells, relatively complete immune reconstitution is achieved.[37]

Patients who receive enzyme replacement therapy (ERT) usually improve immune functions and are well detoxified, but in the long-term, they present with T-cell numbers that are below normal levels and show gradual decline of functional assays, whereas B-cell function defects are not fully repaired, with only 50% of patients able to discontinue Ig replacement therapy.[37] Moreover, a significant part of patients show immune dysregulations, development of antibodies against bovine ADA, and autoimmune manifestations over time.[39–46]

The first gene therapy attempts, aimed to provide treatment for patients lacking an HLA-identical sibling donor, started in the early 1990s, targeting T-lymphocytes from patients on ERT.[47,48] Despite that this approach was not sufficient to discontinue stably ERT, it was shown that the transduced T-cells could safely persist for more than 10 years.[49,50] Importantly, a recently identified population of T-cells with stem cell properties was shown to significantly contribute to the pool of long-term living T-cells in these patients by tracking of insertion sites.[50] Early attempts at gene therapy targeting CD34 progenitor cells were unsuccessful because of a low transduction rate and the decision to keep patients on ERT.[51]

Ameliorations in transduction and protocol changes were introduced in the GT study designs in order to improve the engraftment of modified stem cells, as well as to provide a selective pressure for the corrected cells that would ultimately translate into clinical benefit for the patients.[52] The engraftment of infused stem cells was optimized by the inclusion of a mild preconditioning regimen with busulfan (4 mg/kg i.v.), to make space for the corrected progenitors.[53] Finally, the selective pressure for outgrowth of gene-modified progeny was provided by the withdrawal of ERT before GT. Results obtained at TIGET, Italy, showed in 8/10 treated patients ADA levels sufficient to gain decrease of toxic metabolites and allow functional immune recovery. Thymic activity was restored to normal with polyclonal T-cell receptor repertoires. Normal serum immunoglobulin levels were detected in 50% of patients, allowing for discontinuation of immunoglobulin therapy and production of antibodies after immunization. Importantly, no leukemic or adverse events related to the therapy were observed.[54] A recent update presented at the ESID meeting showed the results of 18 patients treated with an F-U of >1 year, who are all alive; among them, 15 are off-ERT (Table 1).[117]

Subsequently, other patients were treated in London and the United States with a slightly different approach in terms of conditioning regimen and vector design[52] (Table 1). As a result, in 31 out of 42 globally treated patients GT was efficacious, leading to the ERT discontinuation and persistent immune reconstitution, long-term multilineage engraftment, and sustained systemic detoxification.[3,54–56,117,118,122] Furthermore, the study by Candotti et al. compared patients treated with or without chemotherapy confirming the importance of preconditioning on the engraftment of myeloid cells and immune reconstitution.[56] Importantly, the presence of shared vector integrations among multiple hematopoietic lineages demonstrated stable engraftment of multipotent HSC.[57]

Differently from SCID-X1 trial, and despite the use of the same first-generation γ-RV vectors, there were no genotoxic events in GT-treated SCID patients. Integrations were also found in ADA-SCID patients within and/or near potentially oncogenic loci, but did not result in selection or expansion of malignant cell clones *in vivo*[57,58] suggesting that ADA deficiency in itself may create an unfavorable milieu for leukemogenesis. It is important to continue to monitor these patients long-term.

Based on safety issues arisen in clinical trials of retroviral GT for the treatment of other PIDs, alternatively strategies based on ADA encoding LVs were developed. Mortellaro et al. developed an SIN LV in which the expression of the human ADA gene was driven by a PGK promoter. Mice treated with GT early in life were rescued from their lethal phenotype and displayed adequate immune reconstitution and metabolic correction, similar to bone marrow transplantation.[59] To further improve ADA expression, the group of Dr. Kohn and Dr. Gaspar designed an LV that included a codon-optimized human *cADA* gene under the control of the short-form elongation factor-1α promoter (LV EFS ADA) that displayed high-efficiency gene transfer and adequate ADA expression to rescue $ADA^{-/-}$

mice from their lethal phenotype with good T- and B-cell reconstitution. An *in vitro* immortalization assay demonstrated that LV EFS ADA had significantly less transformation potential compared with gRV vectors, without clonal skewing.[60]

On this basis, two phase I/II clinical trials with the use of LV EFS ADA have started in the United Kingdom and the United States for the treatment of ADA-SCID children (Table 1). To date, 5 patients aged between 1.2 and 4.5 years have been treated, after conditioning with busulfan i.v. at a single dose of $\sim$5 mg/kg. At a mean follow-up of about 1 year, there has been significant immunological recovery, with a rise of total T-cell and CD4+ counts and normalization in mitogen responses.[119]

The promising results from gene therapy trials led to issue recommendations from the EBMT Inborn Error Working Party, according to which gene therapy is considered a valid option to all patients without an HLA-identical sibling donor, regardless of the age, availability of an MUD, and outcome of PEG-ADA therapy.[61]

Gene Therapy for WAS

WAS is a rare, complex, X-linked PID disorder caused by mutations in the *WAS* gene[62] characterized by recurrent infections, microthrombocytopenia, eczema, and increased risk of autoimmune manifestations and tumors.[63] The prevalence is estimated to be 1–10 out of a million male individuals, with an incidence of 4 out of a million male live births. The WAS protein (WASp) is a key regulator of actin polymerization in hematopoietic cells[64]; thus, absence or residual WASp expression causes functional defects in different leukocyte subsets, as defective function of T- and B-cells, alteration in NK cell immunological formation synapse, and impaired migration of all leukocyte subsets.[65,66] The life expectancy of WAS patients is severely reduced, unless they are successfully cured by bone marrow transplantation (BMT).[15]

At present, HSCT from HLA-identical sibling donor (MSD) is the treatment of choice for WAS, with a reported 82–88% long-term survival in different European and American centers in the past decade,[67–69] with a survival close to 100% for patients transplanted after year 2000.[15] MUD transplant has reported recently survivals of 85–90%, but better results are obtained when patients are transplanted before the age of 5, and autoimmune complications are more frequent when complete chimerism is not achieved.[67] HSCT from alternative donors (including mismatched family donors and umbilical cord blood) has led to more disappointing results.

In this scenario, therapy with WAS gene-corrected autologous HSCs could represent a valid alternative approach for patients lacking a suitable donor or older than 5 years.[70] Extensive preclinical studies have been performed in the last 15 years to evaluate the feasibility and efficacy of gene transfer by means of both γ-RV[71–74] and LVs.[75,76]

Based on the encouraging results of preclinical studies, a first phase I/II study on humans was conducted since 2007 in Hannover, including 10 patients, treated with WASp-expressing LTR-driven γ-RV following reduced-intensity myeloablation.[77–79] Stable engraftment of gene-corrected cells in multiple lineages (HSCs, lymphoid cells, and myeloid cells) lead to restoration of WASp expression. As previously observed in mixed chimerism preclinical models,[80] a clear proliferative and selective advantage of corrected lymphoid cells over myeloid lineage was also evident in patients. These results were confirmed in a larger cohort of patients, who showed partial to complete resolution of immunodeficiency, autoimmunity, and bleeding.[77,81] However, the analysis of vector common insertion sites revealed a marked clustering between patients, with hotspots found within the proto-oncogenes (LMO2 and MDS/Evi1), already known to be associated in other GT trials with the development of leukemia and myelodysplasia.[3,77,82,83] Between 14 months and 5 years after GT, 7 out of 10 treated patients developed hematologic malignancies.[77,81] These included four cases of T-cell acute lymphoblastic leukemia (T-ALL), two primary T-ALL with secondary acute myeloid leukemia (AML), and one AML, all LMO-2 related. Despite chemotherapy and secondary allogeneic HSCT, two patients died from leukemia.[77] These data indicate that LMO2-driven leukemogenesis is not specific for γc-SCID GT, but it is also seen in WAS GT. The strong viral promoter in the context of an RV, the relatively high vector copy number per cell (1.7–5.2), and the disease background might have contributed to the increased risk of insertional mutagenesis.[77]

While retroviral WAS gene therapy was still at preclinical level, alternative approaches with LVs were developed to overcome the issues related to γ-RV.[84] The own WAS promoter was chosen to drive WASp expression to reduce the risk of insertional oncogenesis and allow a more physiological expression of the transgene. Extensive preclinical studies showed the lack of toxicity in the mouse model of the disease.[85] Moreover, human CD34+ cells were effectively transduced *in vitro* with the vector and engrafted in immunodeficient mice.[86] Clinical trials were then started in Europe and the United States (Table 1), using different conditioning regimens and enrolling patients with severe clinical score and without a suitable BMT donor.[5] Results of the first three patients treated at TIGET have been recently published.[87] After a reduced intensity conditioning with busulfan and fludarabine, patients received autologous HSCs, transduced with the LV encoding the human WASp cDNA. All patients showed a multilineage engraftment of corrected cells, both in bone marrow and peripheral blood compartment, with stable levels of WASp expression.

The immunological function restoration involved T- and B-cell compartment, as well as cytotoxic activity of NK cells and suppressive activity of Treg. Furthermore, platelet counts increased with respect to the pre-GT phase, and platelets presented with normal volume. These biological improvements lead to a clinical benefit for all treated patients, with a reduction of severity and frequency of infections and bleeding and the absence of autoimmune manifestations. The levels of corrected cells in the bone marrow were significantly higher than the engraftment levels achieved in the previous RV trial, suggesting a higher gene transfer efficiency of LV. In terms of safety, analysis of LV insertion profile *in vivo* showed that, in contrast to RV-GT, LV integrations are less prone to cluster near genes involved in hematopoietic functions and potential proto-oncogenes. Moreover, highly represented genes targeted by the vector in these WAS patients were also hit in other LV-GT trials,[88] where no clonal expansion or leukemia have been reported. An SIN-LV configuration and the presence of an autologous

WASp human promoter in the vector construct were crucial in developing a safer GT approach for WAS.[89] A longer evaluation of all patients treated, together with data of other LV-GT trials, will be important to confirm the safety and efficacy of this approach.

Another LV vector using a viral MND-derived promoter has also been used to further increase WASp expression in mice, and results indicate that the γ-RV-derived promoter leads to a stronger transgene expression as compared with the WAS-promoter vector. However, this occurs in association with myeloid clonal expansion and transcriptional dysregulation, highlighting the potential risk of the use of a strong viral promoter.[3,90]

Gene Therapy for CGD

Mutations impairing the expression of gp91phox, p22phox, p47pox, or p67phox molecules are affecting the superoxide production in phagocytic cells, leading to CGD disorder, in which life-threatening abscesses and/or skin, liver, lung, or bone granuloma, and inflammatory complications are characteristic.[23,91] Available therapeutic strategies include antibiotic long-life prophylaxis, IFN-γ administration, and HCT.[23,92] HSCT has recently shown a high success rate as an early intervention in patients with very low superoxide production and in patients with a history of severe invasive fungal infection, organ abscesses, and/or significant inflammatory or autoimmune signs.[17,93] This constitutes an argument in favor of the GT approach for patients without a matched donor. Early clinical trials performed with γRV without conditioning showed only transitory functional correction of $\leq 0.5\%$ of peripheral blood granulocytes.[94,95] Since gene-transduced neutrophils have no survival advantage over defective neutrophils and have a lifespan of only a few days, engraftment of relatively high numbers of gene-transduced HSCs is required by preparatory conditioning.[91]

Most recent trials for X-CGD were conducted in five different centers worldwide (Frankfurt, Zurich, London, NIH, and Seoul) using γRV vector-transduced, mobilized CD34+ cells and nonmyeloablative conditioning with low-dose (8–10 mg/kg) busulfan[93,96–98] ± fludarabine,[92] or melphalan alone (140 mg/m^2)[98] in more than 10 patients. The treatments resulted in initial transient improvement of functional neutrophils up to 30%, with clearance of severe fungal infections and clinical benefit, followed by a yet-unexplained difficulty in achieving long-term engraftment of significant levels of transduced cells, with loss of the expression of the therapeutic gene *gp91phox*.[99] The methylation of the viral promoter leading to silencing of transgene expression is an hypothesis suggested for loss of engraftment.[83]

Alternatively, ectopic gp91phox expression in HSPC could cause the production of reactive oxygen species that may damage DNA, alter cell growth, or induce apoptosis.[100–102] Moreover, immune-mediated mechanisms against gp91phox-expressing cells could have contributed to the lack of long-term persistence.[102] On the other hand, the first-generation γ-RV used in these protocols have also been associated with a high incidence of severe adverse events in the patients with persistent gene marking. A myelodysplastic syndrome (MDS) occurred in three patients (two in Frankfurt, with fatal outcome, and one in Zurich). The second child treated in Zurich displayed a clonal expansion without monosomy 7 or MDS, and this clone disappeared after a successful early HSCT.[93] The frequency of these adverse events highlights the fact that only *gp91phox*-transduced cells with gain-of-function events could persist in patients treated with GT protocols employing LTR-driven RV.[102]

All these events were associated with the insertion near MDS-EVI-1 proto-oncogenes, suggesting the necessity to improve the safety and the efficacy of gene transfer technology.[83,92,97,99,103] At the same time, different strategies to restrict transgene expression to the mature phagocyte compartment were developed using SIN LVs and have been tested in preclinical and clinical development (Table 1). These include gp91phox-encoding vector driven by synthetic chimeric promoter in combination with different myeloid transcription factor binding sites or the A2UCOE element linked to a myeloid promoter driving gp91phox expression in murine myeloid cells.[102,104–106] However, as A2UCOE protects from promoter methylation, its chromatin remodeling properties could have considerable side effects in HSCs,[105,107] and so further studies are needed to proceed to clinical applications.[108]

Another recent approach to improve and maximize transgene expression in myeloid cells while avoiding expression in HSCs is based on the use of an miR-126 target sequence fused to the transgene driven by a myeloid-specific promoter. Transgene expression is provided by the myeloid-specific promoter in myeloid cells and stringent control of gp91phox expression by a miR-126 target sequence in HSCs support further development of this microRNA approach as an alternative gene transfer technique for CGD.[102,106]

A multicentric trial in collaboration between the United Kingdom, Switzerland, and Germany using an LVV with the chimeric promoter was approved and is currently recruiting (Table 1).[109] Preclinical studies for the above dual-regulated LV gene therapy approach are currently ongoing.[109]

New Technologies and Future Plans

The success of gene therapy achieved in the last years has been the result of improved technology and enlarged knowledge on PID and their molecular mechanisms. As the safety of the patients remains a crucial point, the use of new-generation vectors, such as SIN vectors or LVs, showing high efficacy in terms of sustainable transgene expression and reduced risk of insertional mutagenesis tendency *in vitro* and *in vivo*, has been preferred for certain PIDs, characterized by an increased risk of oncogenesis for their genetic background.

Progresses in vector design and HSC biology are favoring the extension of clinical trials to several PID variants, particularly to some challenging ones, for which the current available technologies are not sufficient. Preclinical experiments are ongoing for PIDs, such as Artemis deficiency, CD3γ deficiency, JAK3-SCID, LAD-1, PNP deficiency, RAG1/2 deficiency, X-HIM, XLA, XLP, ZAP70 deficiency, and IPEX, that would benefit from gene therapy approaches.[23]

Furthermore, these results have now been translated from PIDs to other blood-borne disorders, such as lysosomal storage disorders, (β)-thalassemia, and sickle cell disease,

which require a higher therapeutic threshold. Clinical trials with β globin lentivirus vectors are now open at multiple sites, and transfusion independence following GT has been reported in one patient with β-thalassemia.[110] In the metabolic diseases field, gene therapy has led to successful *ABCD1* gene transfer by LV in autologous engineered cells of patients with X-linked adrenoleukodystrophy[111] and stable LV ARSA gene replacement in patients with metachromatic leukodystrophy.[88]

On the other hand, BMT has become much safer and more successful over time for PID patients, thanks to early diagnosis, also because of newborn screening programs, and to the improved outcome of transplants from MUDs determined by new conditioning regimens, accuracy of typing, and new cells manipulation processes. The long-term benefits, safety, and cost-effectiveness of gene therapy versus allogeneic BMT should be evaluated thoroughly in the next years, together with practical issues, such as the choice of vector, the patient's bone marrow stem cell reservoir, and the manufacturing ability to transduce a high number of HSCs.

Gene editing will represent a further step to provide a correction in the defective genes at their genomic locus, maintaining appropriate regulatory control of gene expression and reducing the risk of genotoxicity through ectopic vector insertion. Zinc finger nucleases (ZFNs), meganucleases (MN), transcription activator-like effector nucleases (TALENS), and, more recently, clustered, regularly interspaced, short palindromic repeat (CRISPR) nucleases are all being developed to create highly specific gene targeting.[112–116] The efficiency of gene editing using these techniques has been shown in cell lines and certain primary cell lineages, although remains limited in primary HSCs.[91] Proof of principle for the γ-chain gene has been recently obtained *in vitro* and in animal models.[112]

In conclusion, gene therapy for PID is quickly moving from being an experimental approach to a standard cellular therapy, as demonstrated by the adoption of vector manufacture by mainstream pharmaceutical companies, on the basis of the encouraging results. Further refinement and standardization of the technology will be important for the future clinical development and to enter into the arena of approved therapies.

Author Disclosure Statement

M.P.C. and A.A. declare that no competing financial interests exist.

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